JOURNAL OF AGRICULTURAL RESEARCH

VOL. 65 WASHINGTON, D. C., NOVEMBER 1, 1942 No. 9

AWN INHERITANCE IN BARLEY[1]

By JAMES L. MYLER[2]

Associate in agronomy, *California Agricultural Experiment Station*

INTRODUCTION

Awn inheritance in barley has long been of interest to barley geneticists. The awn or lemma projection on the barley spikelet shows many variations. Morphologically it is an extention of the vascular system of the lemma into a pointed appendage, which varies in different varieties from several inches in length to complete absence. These variations give rise to the various awn types, long-awned, short-awned, awnletted, and awnless. Most so-called awnless varieties, such as Arlington Awnless and Englawnless, do show some awn development, particularly on the central spikelets. In some varieties the central spikelet is awned while the lateral spikelets are awnless, as in most two-row and intermediate (*Hordeum intermedium*) varieties. In six-row varieties the awns on the lateral spikelets are shorter than the central awns; they may be much reduced, or completely absent. Another variation in the lemma projection is the hood, in which the normal awn is replaced by a trifurcate structure. This hood, though commonly sessile, in certain varieties is elevated to varying degrees at the end of an awn.

REVIEW OF LITERATURE

Several investigators have published material on awn inheritance Their reports conflict somewhat, partly, at least, because certain difficulties of classification have tended to obscure the genetics.

Engledow[3] studied rather extensively the awn inheritance in a cross between a fully awned variety and Englawnless. Fluctuations in awn length, caused by environment, complicated the problem of classification. Engledow's awnless variety, when planted late, developed up to one-half awns, whereas single, late-forming heads on plants otherwise completely awnless often produced one-fourth to one-half awns. From F_2 data Engledow concluded that awned vs. awnless must be due to two factors; but on checking in F_3 he concluded that only one factor was involved.

Kuckuck,[4] in reviewing the literature, cities work of Huber, who attributed awned vs. awnless to a single factor, the F_1 being short awned, the F_2 segregating 1 long-awned: 2 short-awned: 1 awnless.-Ubisch[5] reports a two-factor difference between long-awned and awnless, the factor *J* producing long awns, the recessive *j* producing short

[1] Received for publication November 26, 1941.

[2] Thanks are due Dr. F. N. Briggs for furnishing material, advice, and guidance throughout the experiments and during the preparation of this paper, and also to Dr. E. H. Stanford for valuable help in classifying and analyzing the results.

[3] ENGLEDOW, F. L. INHERITANCE IN BARLEY. III. THE AWN AND LATERAL FLORET: FLUCTUATION: A LINKAGE: MULTIPLE ALLELOMORPHS. Jour. Genet. 14: [49]-87, illus. 1924.

[4] KUCKUCK, H. DIE GENETIK DER GERSTE. (Sammelreferat) Zuchter 2: 50-60, illus. 1930.

[5] UBISCH, G. v. BEITRAG ZU EINER FAKTORENANALYSE VON GERSTE. Deut. Bot. Gesell. Ber. 41: 79-84. 1923.

awns. A second factor *S* causes awnless, though Ubisch makes no statement concerning the interaction of *J* and *S*.

Miyake and Imai [6] (Complete translation by Hiro Taketaya, senior Japanese student at the University of California) encountered much the same difficulty of classification as did Engledow. Their awnless variety developed short awns on late tillers. They reported a single-factor difference between long and short awns; a three-factor difference between a long-awned six-rowed variety and an awnless six-rowed variety. Although they made three classes—long-awned, short-awned, and awnless—the awn types were bulked to show the three-factor difference. In a cross of their awnless six-rowed variety with a long-awned two-rowed variety, they concluded that the two-rowed variety had four dominant factors A_1, A_2, A_3, A_4, for awn development, whereas the six-rowed awnless variety carried the recessives, a_1, a_2, a_3, a_4. Here, again, all awn types were bulked, though the investigators made a separation of the classes, long-awned, short-awned, and awnless. No F_3 generation was grown to check F_2 phenotypes.

Many workers [7] have reported hooded vs. awned as due to a single factor. Miyake and Imai [8] attributed it to a single factor; but hooded vs. awnless in a cross of their six-rowed awnless with a two-rowed hooded was reported as due to complementary factors. In this cross the F^2 segregation was 9 hooded: 7 normal. The normal would presumably be awnless, but was not described by them. From the author's experience (presented later), the segregates in F_2 from a cross of awnless with hooded would include, besides hooded and awnless, three awn lengths.

MATERIALS

Four long-awned, one awnless, and one hooded variety of barley (*Hordeum vulgare* L.) have been used in crosses thus far. The study of crosses involving other awn types is under way.

Atlas, the most important commercial variety of California, is a selection from Coast. It is a long-awned, six-rowed, lax type. Black Hull-less is a long-awned, six-rowed, lax, purple, naked type. An unnamed variety, C. I. No. 5628, is also a long-awned, six-rowed, lax purple, naked type. Another variety, Redrachis, C. I. No. 5649, is a two-rowed, lax type, with long smooth awns and a red rachis. Awnless, C. I. No. 5631, is an Atrada selection brought in by Dickson in 1932 from Russia. This variety is truly awnless, developing no awns, regardless of planting date or environmental factors at Davis, Calif. In addition, it is quite dense, has a short, stiff straw and matures fairly early. Nepal, a hooded, six-rowed, lax, naked variety, was the parent used in studying hooded vs. awnless. Although these varieties differ in other characters, the contrasting characters reported in this paper are shown in table 1.

[6] MIYAKE, KICHI, and IMAI, YOSHITAKA. GENETIC STUDIES IN BARLEY. I. Bot. Mag. [Tokyo] 36: 25-38. 1922. [In Japanese. English resumé, p. 27.]

[7] ROBERTSON, D. W., WIEBE, G. A., and IMMER, F. R. A SUMMARY OF LINKAGE STUDIES IN BARLEY Amer. Soc. Agron. Jour. 33: 47-64. 1941.

[8] See footnote 6.

TABLE 1.—*Parent varieties used in the study of awn inheritance*

Parent variety	C. I.[1] No.	Contrasting characters		
		Awn or hood type	Density	Rows
Awnless	5631	Awnless	Dense	6
Atlas	4118	Long-awned	Lax	6
Black Hull-less	666	do	do	6
Unnamed	5628	do	do	6
Redrachis	5649	do	do	2
Nepal	595	Hooded	do	6

[1] C. I. denotes accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry U. S. Department of Agriculture.

EXPERIMENTAL RESULTS

AWNED × AWNLESS

The awn types as classified in F_2 of the cross Atlas × Awnless, showed 351 awned, 122 awnletted, and 32 awnless, which suggests a 12:3:1 ratio, although the fit is not satisfactory. The awnletted segregates had short awns on the central spikelets; and, though they varied somewhat, they tended to approach the awn type of Arlington Awnless. A two-factor basis for awn inheritance is further indicated by 473 awned: 32 awnless, where 473.4: 31.6 were expected on the basis of a 15: 1 ratio, and thus almost a perfect fit. F_3 rows of about 30 plants each were grown, from which the F_2 genotypes were determined.

The classification system was expanded to take care of the many breeding types encountered in F_3 and to improve on the F_2 classification, which, as pointed out above, was not entirely satisfactory. In view of the experience with awn shedding encountered in classifying F_2 plants, the F_3 generation was classified while the plants were still green. A close study in F_3 revealed that plants formerly classed as long-awned could be divided into long- and short-awned. This had been overlooked in F_2 because the actual length is influenced by head density (fig. 1). In very few cases were the lax plants as awnless as the awnless parent. The F_3 classification showed that the nine genotypes expected on the basis of two factors were present in about the expected numbers (table 2). The four homozygous types—long-awned, short-awned, awnletted, and awnless—are shown in figure 1.

TABLE 2.—*Awn segregation in F_3 rows of Atlas × Awnless, grown at Davis, Calif., in 1939*

Type of segregation	Genotype	Observed	Expected	χ^2
Long-awned	$LkLk\ Lk_1Lk_1$	43	31.5	4.198
3 long-awned: 1 short-awned	$LkLk\ Lk_1lk_1$	65	63.0	.063
3 long-awned: 1 awnletted	$Lklk\ Lk_1Lk_1$	57	63.0	.571
All types	$Lklk\ Lk_1lk_1$	130	126.0	.127
Short-awned	$LkLk\ lk_1lk_1$	32	31.5	.008
3 short-awned: 1 awnless	$Lklk\ lk_1lk_1$	53	63.0	1.587
Awnletted	$lklk\ Lk_1Lk_1$	42	31.5	3.500
3 awnletted: 1 awnless	$lklk\ Lk_1lk_1$	55	63.0	1.016
Awnless	$lklk\ lk_1lk_1$	27	31.5	.643
Total		504	504.0	11.713

The chi-square for the data in table 2 is 11.713, which gives a probability slightly below the 20-percent point. The two-factor explanation was further substantiated by a backcross of the F_1 to Atlas, which yielded the table 3 data in F_1 as determined from the segregation in F_2 rows.

FIGURE 1.—The four true breeding awn types, long-awned (*A*), short-awned (*B*), awnletted (*C*), and awnless (*D*), as they appear on the lax and dense spikes, respectively.

TABLE 3.—*Awn segregation in F_2 of the backcross of Atlas × Awnless to Atlas, grown at Davis, Calif., in 1939*

Type of segregation	Genotype	Observed	Expected	χ^2
Long-awned	*LkLk* Lk_1Lk_1	6	7.75	0.395
3 long-awned: 1 short-awned	*LkLk* Lk_1lk_1	8	7.75	.008
3 long-awned: 1 awnletted	*Lklk* Lk_1Lk_1	9	7.75	.202
All types	*Lklk* Lk_1lk_1	8	7.75	.008
Total		31.0	31.00	.613

The four classes (table 3) expected in the backcross should occur in equal numbers. The chi-square value of 0.613 shows an excellent fit to the expected, with a probability above 0.8. Atlas differs, therefore, from Awnless in two major dominant factors (*LkLk* Lk_1Lk_1) for awn development, both of which are necessary for the development of long awns. One dominant, $Lklk_1$, alone produces short awns, whereas the other, $lkLk_1$, gives the awnletted condition.

Additional data concerning awn inheritance are available from three other crosses, grown in part for other purposes. These will be considered briefly.

The first, Awnless × Black Hull-less, as classified in F_2 showed 297 long-awned, 256 short-awned, 237 awnletted, and 39 awnless, where 466.3, 155.4, 155.4, and 51.8, respectively, were the numbers expected on the basis of two factors. Here, again, the poor fit was due to difficulties in classification. The awn in Balck Hull-less is somewhat longer than in Atlas although both are considered long-awned. The heterozygous types in this cross tended to be somewhat more intermediate than in the Atlas cross. Plants heterozygous for both factors were classified about equally in the long- and short-awn classes, a few being classified as awnletted, as later shown by the F_3. Many plants heterozygous for long-awned and awnletted (*Lklk*, Lk_1Lk_1) were classified as short-awned; and plants of the genotype (*Lklk*, lk_1lk_1) which in F_3 segregate 3 short-awned: 1 awnless were often classified as awnletted.

Four hundred rows of the F_3 generation of this cross were grown and classified. The F_2 generation of those 400 plants showed 154 long-awned, 122 short-awned, 108 awnletted, and 16 awnless, where 225, 75, 75, and 25 were expected. This is a very poor fit to the expected 9:3:3:1 ratio. The results form the F_3 classification are shown in table 4, and conform to expectations.

TABLE 4.—*Awn segregation in F_3 rows of Black Hull-less × Awnless, grown at Davis, Calif., in 1941*

Type of segregation	Genotype	Observed	Expected	χ^2
Long-awned	*LkLk* Lk_1Lk_1	34	25	3.240
3 long-awned: 1 short-awned	*LkLk* Lk_1lk_1	49	50	.020
1 long-awned: 2 short-awned: 1 awnletted	*Lklk* Lk_1Lk_1	49	50	.020
All types	*Lklk* Lk_1lk_1	103	100	.090
Short-awned	*LkLk* lk_1lk_1	29	25	.640
1 short-awned: 2 awnletted: 1 awnless	*Lklk* lk_1lk_1	39	50	2.420
Awnletted	*lklk* Lk_1Lk_1			
3 awnletted: 1 awnless	*lklk* Lk_1lk_1	97	100	.090
Awnless	*lklk* lk_1lk_1			
Total		400	400	6.520

The chi-square value of 6.52 shows a probability of between 0.3 and 0.5. Thus the data in table 4 show clearly that in this, as in the Atlas cross, awn inheritance depends upon two factors. Plants heterozygous for long-awned and awnletted, though usually classified as short-awned, may be distinguished from homozygous short-awned plants because the awns on the central spikelets are coarser and tend to be much longer than the awns on the lateral spikelets.

When the F_3 data are used to correct the F_2 classification, there are found to be 235 long-awned: 68 short-awned: 80 awnletted: 17 awnless, where 225: 75: 75: 25 are expected, giving a P value greater than 0.2.

An F_2 population of 496 plants of C. I. No. 5628 × Awnless was classified in 1941 for awn inheritance. The author, having become more familiar with the heterozygous types from classifying the F_3 rows of Black Hull-less × Awnless, was able to effect a satisfactory classification in F_2 of this cross, making it unnecessary to grow the F_3. The F_2 data are shown in table 5.

TABLE 5.—*Awn segregation in F_2 of the cross C. I. No. 5628 × Awnless, grown at Davis, Calif., in 1941*

Phenotype	Observed	Expected	χ^2
Long-awned	273	279	0.129
Short-awned	99	93	.387
Awnletted	99	93	.387
Awnless	25	31	1.161
Total	496	496	2.064

With a probability between 0.5 and 0.7, the data in table 5 agree with those for the two previously discussed crosses; and apparently C. I. No. 5628 carries the same awn factors as Atlas and Black Hull-less.

An F_2 population of 352 plants in the cross of two-rowed, long-awned Redrachis × Awnless was grown in 1941. Since the heterozygous types must be identified from the difference in length of central and lateral spikelets, it was not possible in this cross to separate these types. The F_2 plants were classified, accordingly, as awned or awnless; this system showed 323 awned: 29 awnless, giving a chi-square value of 2.375 and a probability between 0.1 and 0.2 for a 15:1 ratio. These results do not agree with the results of Miyake and Imai,[9] who showed a four-factor difference between a six-rowed awnless and a two-rowed awned variety. The varieties used by them, however, are not the same varieties used in this study.

HOODED × AWNLESS

In the cross, hooded Nepal × Awnless, the F_2 segregation shows all the awn types that occur in the cross awned × awnless, in addition to hooded segregates.

The same difficulties were encountered in classifying this cross as in the previous crosses, density again affecting awn length. The awnletted and awnless types were combined because of the difficulty of classification, particularly in F_2 populations. An F_2 population of 882 plants yielded the results shown in table 6.

TABLE 6.—*Segregation for hooded, awned, and awnless in the cross Nepal × Awnless, grown at Davis, Calif., in 1940*

Phenotypes	Dominant genes	Observed	Expected	χ^2
Hooded	*LkLk*$_1$ *K*	372	372.1	0.000
Long-awned	*LkLk*$_1$ *k*	109	124.0	1.815
Short-awned	*Lklk*$_1$ (*K*, or *k*)	162	165.4	.070
Awnletted	*lkLk*$_1$ (*K*, or *k*)	239	220.5	1.552
Awnless	*lklk*$_1$ (*K*, or *k*)			
Total		882	882.0	3.437

The chi-square for the data in table 6 is 3.437, which gives a probability between 0.3 and 0.5. This explanation assumes that both factors for awn development must be present before a hood can develop, and agrees with the previous explanation for awn inheritance. In this cross, Nepal carries both factors for awn development in addition to

[9] See footnote 6.

the hood factor, which masks awn development. This explanation differs from the results reported by Miyake and Imai [10] from a cross of their six-rowd awnless × two-row hooded. They report complementary factors for hooded vs. normal, with hooded dominant.

The F_3 generation of this cross was planted in the fall of 1940, and 416 rows were classified in the spring of 1941. Table 7 shows the results of the F_3 classification.

TABLE 7.—*Segregation in F_3 rows for hooded, awned, and awnless in the cross Nepal × Awnless, grown at Davis, Calif., in 1941*

Type of segregation	Genotype	Observed	Expected	χ^2
Hooded	*LkLk Lk$_1$Lk$_1$ KK*	8	6.5	0.346
3 hooded: 1 long-awned	*LkLk Lk$_1$Lk$_1$ Kk*	14	13.0	.077
3 hooded: 1 short-awned	*LkLk Lk$_1$lk$_1$ KK*	16	13.0	.692
3 hooded: 1 awnletted	*Lklk Lk$_1$Lk$_1$ KK*	20	13.0	3.769
9 hooded: 3 long-awned: 4 short-awned	*LkLk Lk$_1$lk$_1$ Kk*	12	26.0	7.538
9 hooded: 3 long-awned: 4 awnletted	*Lklk Lk$_1$Lk$_1$ Kk*	16	26.0	3.846
9 hooded: 3 short-awned: 3 awnletted: 1 awnless	*Lklk Lk$_1$lk$_1$ KK*	38	26.0	5.538
27 hooded: 9 long-awned: 12 short-awned: 12 awnletted: 1 awnless.	*Lklk Lk$_1$lk$_1$ Kk*	50	52.0	.077
Long-awned	*LkLk Lk$_1$Lk$_1$ kk*	15	6.5	11.115
3 long-awned: 1 short-awned	*LkLk Lk$_1$lk$_1$ kk*	22	13.0	6.231
3 long-awned: 1 awnletted	*Lklk Lk$_1$Lk$_1$ kk*	17	13.0	1.231
9 long-awned: 3 short-awned: 3 awnletted: 1 awnless.	*Lklk Lk$_1$lk$_1$ kk*	25	26.0	.038
Short-awned	*LkLk lk$_1$lk$_1$* { *KK* / *Kk* / *kk* }	30	26.0	.615
3 short-awned: 1 awnless	*Lklk lk$_1$lk$_1$* { *KK* / *Kk* / *kk* }	39	52.0	3.250
Awnletted	*lklk Lk$_1$Lk$_1$* { *KK* / *Kk* / *kk* }	29	26.0	.346
3 awnletted: 1 awnless	*lklk Lk$_1$lk$_1$* { *KK* / *Kk* / *kk* }	43	52.0	1.558
Awnless	*lklk lk$_1$lk$_1$* { *KK* / *Kk* / *kk* }	22	26.0	.615
Total		416	416.0	46.882

The chi square for the data in table 7 shows a poor fit to the expected, with a *P* value below 0.01. The homozygous long-awned rows, about which there could be little chance for error in classification, contribute almost one-fourth of the total chi square. The number of plants per row was too small for accurate determination of such ratios as 9 hooded: 3 long: 4 short; but the number of rows obtained is reasonably close to that expected. When the F_2 was classified on the basis of F_3, there were 174 hooded, 79 long-awned, 69 short-awned, 72 awnletted, and 22 awnless, where 175.5, 58.5, 78.0, 78.0, and 26.0 were expected if Nepal carries both awn factors in addition to the hooded factor. This gives a chi-square value of 9.312 and a *P* value between 0.05 and 0.1.

As the F_2 and F_3 data clearly show, Nepal differs from Awnless in in the three factors *LkLk*, *Lk$_1$Lk$_1$*, and *KK*, and both dominant awn factors must be present for the expression of hoods. In several cases, however, a "rudimentary hood" developed on the short-awned and awnletted plants. In the short-awned plants it took the form of a flat, somewhat wider area in the awn, at which point the awn tended to bend or twist. In the awnletted plants it tended to cause a minute fork on the tip of a few of the awnletted central spikelets. These

[10] See footnote 6.

were not classed as hoods because they did not develop uniformly and were much less numerous than would be expected on the basis of a reasonable genetic interpretation.

SUMMARY

Awn inheritance in the cross Atlas × Awnless was shown to be due to two factors, *Lklk* and Lk_1lk_1. Long-awned plants carry both dominants. Short-awned plants have the first factor, *Lk*, dominant and the second, Lk_1 recessive. Awnletted plants have the first factor, *Lk*, recessive and the second, Lk_1, dominant. Plants carrying both recessives are awnless. This two-factor explanation is also true in the crosses Black Hull-less × Awnless and C. I. No. 5628 × Awnless. In the two-rowed, long-awned, Redrachis × Awnless cross, a two-factor difference was shown with a 15:1 ratio.

In the Nepal × Awnless cross it was shown that these varieties differ by three factors for awn development. Nepal carries both awn factors, *LkLk* and Lk_1Lk_1, besides the factor *KK* for hood development. Awnless carries the three recessives. For the development of hooded plants, both dominant awn factors must be present in addition to the hood factor. Thus in F_2 the expected segregation is 27 hooded: 9 long-awned: 12 short-awned: 12 awnletted: 4 awnless. The hood factor, *KK*, in a few cases tends to flatten and to cause a bend or twist near the end of the short awns. It also tends to cause a minute fork at the tip of the awnlet of a few central spikelets in the awnletted segregates.

INSECT TRANSMISSION OF THE VIRUS CAUSING NARCISSUS MOSAIC[1]

By F. S. BLANTON, *assistant entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration United States Department of Agriculture,* and F. A. HAASIS, *assistant professor of plant pathology, New York (Cornell) Agricultural Experiment Station, and collaborator, Bureau of Plant Industry, Agricultural Research Administration United States Department of Agriculture*

INTRODUCTION

The mosaic disease of narcissus is known to be caused by a transmissible virus (*9*, *12*, *13*),[2] but the method by which the virus is disseminated has remained obscure. Several theories have been advanced, including the possibility of insects being either directly or indirectly concerned. Darlington (*7*) suggested that the disease may be caused through root injury resulting from larval feedings of the swift moth. Hodson (*10*) considered *Thrips tabaci* Lind. to be a likely vector of the virus, but according to Smith (*14*, *p. 391*), Hodson later claimed that evidence is against such an hypothesis and considered *Tarsonemus laticeps* Halbert (*T. approximatus* var. *narcissi* Banks) as worthy of consideration as a vector. In a later report Hodson (*11*) mentioned this mite as well as the mosaic disease of narcissus, but said nothing of the mite's being a probable vector of the virus; so presumably the theory was abandoned.

Following a field survey on Long Island, Blanton (*2*) reported that 20 species of leafhoppers and related Homoptera had been observed feeding on narcissus. In another paper (*3*) he listed 5 species of thrips from narcissus. Subsequently the clover mite (*Eryobia praetiosa* Koch) and the springtail *Bourletiella hortensis* (Fitch) were found on this plant. In addition, 7 species of aphids feeding on narcissus foliage have been collected—*Macrosiphum solanifolii* (Ashm.), *M. taraxaci* (Kalt.), *M. pisi* (Kalt.), *Aphis rumicis* L., *Myzus persicae* (Sulz.), *Anuraphis roseus* (Baker), and *Brevicoryne brassicae* (L.).

Several workers (*1*, *6*, *11*, *12*) have questioned the association of aphids, the usual vectors of mosaic pathogens, with the natural dissemination of the virus causing narcissus mosaic. The present writers (*4*, *5*), however, reported in recent notes that the aphid species *Macrosiphum solanifolii*, *M. pisi*, *M. rosae* (L.), *Aphis rumicis*, *Myzus convolvuli* (Kalt.), *M. cerasi* (F.), and *Anuraphis roseus* are capable of transmitting the virus. It is the purpose of this paper to present a detailed account of this work as well as of experiments with other possible vectors, including several species of thrips and leafhoppers, the springtail previously mentioned, the clover mite, and the bulb scale mite (*Tarsonemus laticeps*).

[1] Received for publication December 30, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 419.

MATERIALS AND METHODS

Several varieties of virus-free narcissus plants were used in the transmission tests. All these plants were grown on the laboratory grounds for 2 or more years before being used in the experiments. During this period the plants were inspected early in the season, and all plants that appeared to be infected were removed. Mosaic-infected Sir Watkin plants served as the source of virus inoculum for all the tests. After being used for this purpose, they were isolated and grown for another year to check the first diagnosis.

With the exception of *Frankliniella fusca* (Hinds), a stock colony of which was obtained from J. G. Watts of the South Carolina Agricultural Experiment Station, all insect and mite species were collected from various host plants in the vicinity of narcissus plantings in Babylon, N. Y. These collections also served as the source for some of the aphid species established in pure-line colonies, which were reared after single aphids had been manually transferred to suitable host plants confined in cheesecloth cages.

Tests were conducted both in the greenhouse and in the field. Prior to 1935 healthy test plants were grown singly in 8-inch porous-clay pots in the greenhouse, to avoid the possibility of virus transfer by root contact. Haasis (*9*), however, demonstrated from experiments conducted in 1934 that virus transfer in this manner was unlikely; so in 1935 and subsequent years healthy and mosaic plants were grown together in either pots or flats, or in field plots. Each flat and each field plot contained from 20 to 24 plants in the ratio of approximately 1 diseased plant to 4 healthy plants, and the flats and plots were sometimes replicated several times.

Most of the plants grown in pots were enclosed in celluloid cylinders according to technique described elsewhere (*9*). For tests with the bulb scale mite, pots containing a diseased and a healthy plant were placed in a greenhouse where no other narcissus plants were being grown and were isolated from each other by means of a water barrier. The flats and field plots were enclosed in cages covered with fine-mesh cheesecloth.

In the tests with plants grown singly in pots, the insects were fed upon the mosaic-diseased plants for a definite period and then transferred to the cages containing the healthy plants. As a check, insects of the same species were fed for the same time on healthy narcissus and then transferred to other healthy narcissus. In the tests with diseased and healthy plants confined together, the insects were transferred to these plants directly from the host plant. All tests with aphids (with one exception as shown in table 1), thrips, and one mite were handled in this manner. This mite and the thrips were allowed to remain until all the plants died, being free to feed on both healthy and diseased plants. These species increased enormously, several generations being involved. The life cycle was complete, all stages being present when the plants died. All the aphids, however, died a few days after being confined with narcissus. A number of field plots and some flats of bulbs were caged, and thus kept free of insects, to serve as a second set of checks in some of the transmission tests involving aphids.

RESULTS

The results of the transmission tests with miscellaneous insects and mites are given in tables 1 and 2, and those with aphids in table 3. The data for the check flats and plots from which insects were excluded are given in table 4.

TABLE 1.—*Transmission of the virus causing narcissus mosaic by insects and mites when transferred from mosaic plants, and from healthy plants, to individually potted healthy Sir Watkin narcissus plants, 1932–34*

Species	Host plant	Tests with insects transferred from mosaic to healthy plants				Tests with insects transferred from one healthy plant to another (checks)			
		Time confined before transfer to healthy plants	Time confined on healthy plants	Insects per plant	Healthy plants exposed	Plants developing infection	Time confined after transfer	Insects per plant	Plants exposed	Plants developing infection
		Days	*Days*	*Number*	*Number*	*Number*	*Days*	*Number*	*Number*	*Number*
Aceratagallia sanguinolenta (Prov.)	Clover (*Trifolium* sp.).	¾	15	10	5	0	15–18	10	5	0
Macrosteles divisus (Uhl.).	Ragweed (*Ambrosia* sp.).	3	5	10	5	0	8–10	10	5	0
Dikraneura sp.	Quackgrass (*Agropyron repens* (L.) Beauv.	3	3–6	10	5	0	4–9	10	5	0
Dracculacephala mollipes (Say).	do	2	1–2	10	5	0	3	10	5	0
Stobaera tricarinata (Say).	Ragweed. (*Ambrosia* sp.).	2	4–6	3–6	5	0	3–6	3–6	5	0
Bourletiella hortensis (Fitch).	Clover (*Trifolium* sp.).	2–3	5	15–20	5	0	5	15–20	5	0
Bryobia praetiosa Koch.	Quackgrass (*Agropyron repens*).	2–3	5	10–20	5	0	2–10	10–20	5	0
Aphis rumicis L.	Dock (*Rumex* sp.)	2	3	Many	5	3	3–6	Many	5	0

TABLE 2.—*Transmission of the virus causing narcissus mosaic by thrips and mites when confined throughout their life cycle with diseased and healthy Sir Watkin narcissus plants potted together, 1935*

Species	Host plant	Insects confined with a mosaic and a healthy plant			Insects confined with healthy plants only (checks)		
		Insects per plant	Healthy plants exposed	Plants developing infection	Insects per plant	Healthy plants exposed	Plants developing infection
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
Frankliniella fusca (Hinds).	Cotton (*Gossypium* sp.)	10–15	29	0	50	5	0
Thrips tabaci Lind.	Onion (*Allium* sp.)	5–25	51	0	3–20	19	0
Tarsonemus laticeps Halbert.	Narcissus *Narcissus pseudonarcissus* L.).	Many	3	0			

[1] Insects remaining through several generations, the life cycle being complete.

TABLE 3.—*Transmission of the virus causing narcissus mosaic by 7 species of aphids when confined with plants in flats or field plots, 1935–39*

Species	Host plant on which aphids developed	Variety of narcissus	Location of plants	Date of transfer of aphids to healthy plants	Tests with aphids transferred from mosaic to healthy plants		Tests with aphids transferred from one healthy plant to another (checks)	
					Plants exposed	Plants developing infection	Plants exposed	Plants developing infection
					Number	*Number*	*Number*	*Number*
Macrosiphum solanifolii (Ashm.) [1]	Potato (*Solanum tuberosum* L.)	Sir Watkin	Greenhouse	Feb. 2	70	14	23	0
				Feb. 10	72	32	24	0
				Feb. 17	70	27	46	1
				Mar. 4	71	29	47	2
		King Alfred	do	Mar. 9	24	24	20	1
		Sir Watkin	Field	May 1–10	58	18	53	5
		Minister Talma	do	do	38	8	17	1
		Subtotal			403	152	230	10
Macrosiphum rosae (L.)	Rose (*Rosa* sp.)	Sir Watkin	Field	May	20	12		
		King Alfred	do	May 1–10	58	36		
		Minister Talma	do	May	58	41	54	2
		Subtotal			136	89	54	2
Macrosiphum pisi (Kalt.)	Vetch (*Vicia* sp.)	Sir Watkin	Field	May 1–10	31	24		
Aphis rumicis L.	Nasturtium (*Tropaeolum* sp.) [1] or dock (*Rumex* sp.)	Sir Watkin	Greenhouse	Feb. 2	21	5		
			do	Feb. 10	22	8	23	1
			do	Apr. 30	277	240	119	8
			Field	May 1–10	58	40	40	3
		Minister Talma	do	do	39	12	17	2
		King Alfred	do	do	80	46		
		Spring Glory	Greenhouse	May	24	6	15	0
		Victoria	do	do	84	41	12	0
		Subtotal			605	398	226	14
Myzus convolvuli (Kalt.)	Tulip (*Tulipa* sp.)	Sir Watkin	Greenhouse	Mar. 4	48	25	20	4
			do	Mar. 30	233	157	96	3
		King Alfred	do	Mar. 9	43	28	24	2
		Subtotal			324	210	140	9
Myzus cerasi (F.)	Cherry (*Prunus* sp.)	Sir Watkin	Field	June 5	36	15		
Anuraphis roseus (Baker)	Apple (*Malus* sp.)	do	do	June 1	18	13		
		Total			1,553	901	651	35

[1] Pure-line colonies.

TABLE 4.—*Development of mosaic disease in narcissus plants in field plots or flats that were caged to exclude insects, 1935–39*

Variety of narcissus	Location of plants	Plants caged	Plants developing infection
		Number	*Number*
Sir Watkin	Field	106	4
King Alfred	do	74	5
Victoria	Greenhouse	51	6
	do	12	0
Total		243	15

In the early tests with miscellaneous insects negative results were obtained with all species except *Aphis rumicis*. Since this species transmitted the virus to three of the five healthy narcissus plants exposed to inoculation, and since the check plants remained healthy, all transmission trials are considered as comparable. In the later tests with aphids all seven species transmitted the mosaic virus, but there was considerable variation in the proportion of plants infected.

A few of the check plants from which insects were excluded also became infected, but the number was very small compared with that of plants containing aphids. The cause of these accidental infections is uncertain, but two possibilities are suggested: (1) Some of the supposedly healthy plants on which the colonies were maintained may have served as symptomless carriers of the virus; (2) migratory aphids, having acquired the virus by feeding on mosaic narcissus growing near the cages, may have made a feeding contact with the healthy foliage through the cheesecloth barriers. The writers believe the second possibility to be the more tenable, for they did observe a few migratory aphids feeding through the cheesecloth. Furthermore, 18 of the 22 accidental transmissions occurring in the caged field plots were found to be in plants next to the periphery of the cage and the remaining 4 were in the adjacent row, approximately 10 inches from the edge of the cage. If these accidental transmissions resulted from inoculations within the cages, a more random distribution of the infected plants should be expected. The locations of infected plants in the greenhouse were not charted, but the majority of the accidental transmissions occurred in plants adjacent to the edge of the cage.

A total of 904 plants were infected as a result of inoculation by aphids, but none showed morphologic symptoms of the mosaic disease during the growing season in which the inoculations were made. Similar results are reported where inoculations were performed mechanically (*9*, *12*, *13*).

The experimentally infected plants used in these tests expressed both the chlorotic-striping and leaf-enation symptoms that are characteristic of the disease as exhibited by naturally infected plants of the same variety growing in the field.

DISCUSSION

Narcissus plantings in Long Island are first invaded by aphids in the latter part of April, when these insects commence their migrations

[1] Pure-line colonies.

from primary to secondary hosts, and from this early date until the narcissus plants mature light populations persist. It appears that narcissus serves only as a transient host for the aphids, for under controlled conditions, when aphids are restricted to a narcissus diet while the plants are still growing, the period of survival ranges from 3 to 15 days. The species *Macrosiphum solanifolii*, however, is exceptional in that it forms colonies and multiplies rapidly on narcissus foliage, beginning the latter part of June.

Of the seven aphid species that have been taken on narcissus in the field, the three most frequently encountered have been *Macrosiphum solanifolii*, *M. pisi*, and *Aphis rumicis; Anuraphis rosae* was abundant only one year during these investigations. All four species transmitted the virus under experimental conditions (table 3). Three additional species, *M. rosae*, *Myzus convolvuli*, and *M. cerasi*, although never collected from narcissus, are also efficient vectors. These facts suggest that the virus causing narcissus mosaic may be disseminated by many species of aphids, as has similarly been demonstrated for the yellow dwarf virus of onions (*8*).

In most of the transmission tests with aphids from 100 to 500 insects per cage were employed. With the exception of *Macrosiphum solanifolii* late in the growing season, aphid populations on narcissus under field conditions rarely if ever reach such high numbers. Preliminary data are at hand, however, which indicate that a single aphid is able to transmit the virus. In such a case even sparse populations could account for the high percentage of virus dissemination that takes place in the field.

SUMMARY

Investigations were conducted to determine whether any of the insects collected from field-grown narcissus plants are capable of transmitting the virus causing narcissus mosaic.

Fifteen species of insects and two species of mites were allowed to feed on mosaic narcissus plants and were then transferred to healthy narcissus, which were maintained in cages in both the greenhouse and the field. The results were negative for the miscellaneous insects and mites, which included five species of leafhoppers, *Aceratagallia sanguinolenta* (Prov.), *Macrosteles divisus* (Uhl.), *Dikraneura* sp., *Draeculacephala mollipes* (Say), *Stobaera tricarinata* (Say); two species of thrips, *Frankliniella fusca* (Hinds) and *Thrips tabaci* Lind.; one springtail, *Bourletiella hortensis* (Fitch); and two species of mites, *Bryobia praetiosa* Koch and *Tarsonemus laticeps* Halbert. All seven species of aphids—*Macrosiphum solanifolii* (Ashm.), *M. rosae* (L.), *M. pisi* (Kalt.), *Aphis rumicis* L., *Myzus convolvuli* (Kalt.), M. *cerasi* (F.), and *Anuraphis roseus* (Baker)—gave positive results.

Four of the aphid species—*Macrosiphum solanifolii*, *M. pisi*, *Anuraphis roseus*, and *Aphis rumicis*—have been collected on narcissus plants growing in the field, but only *M. solanifolii* has been found capable of multiplying on these plants.

The aphids transmitted the virus to 904 out of a total of 1,558 plants of the following narcissus varieties: Sir Watkin, King Alfred, Minister Talma, Spring Glory, and Victoria.

Symptoms of the disease appeared during the season following inoculation and were typical of the disease as exhibited by naturally infected plants of the same variety growing in the field.

LITERATURE CITED

(1) Atanasoff, D.
1928. mosaic disease of flower bulb plants. B'lgarsk. Bot. Druzh. Izv. (Soc. Bot. de Bulgarie, Bul.) 2: [51]–60, illus.
(2) Blanton, F. S.
1937. leafhoppers and homoptera of related families collected in and adjacent to narcissus plantings. (Scientific Note) Jour. Econ. Ent. 30: 972.
(3) ———
1939. notes on some thrips collected in the vicinity of babylon, long island, n. y. N. Y. Ent. Soc. Jour. 47: 83–94.
(4) ——— and Haasis, F. A.
1939. transmission of the narcissus mosaic virus by aphids. (Scientific Note) Jour. Econ. Ent. 32: 469–470.
(5) ——— and Haasis, F. A.
1940. three additional species of aphids transmitting narcissus mosaic. (Scientific Note) Jour. Econ. Ent. 33: 942.
(6) C[hittenden], F. J.
1933. mosaic disease of narcissi. Roy. Hort. Soc. Daffodil Yearbook 1933, pp. 72–73.
(7) Darlington, H. R.
1908. yellow-stripe in daffodils. Roy. Hort. Soc. Jour. 34: [161]–166.
(8) Drake, C. J., Tate, H. D., and Harris, H. M.
1933. the relationship of aphids to the transmission of yellow dwarf of onions. Jour. Econ. Ent. 26: 841–846, illus.
(9) Haasis, F. A.
1939. studies on narcissus mosaic. N. Y. (Cornell) Agr. Expt. Sta. Mem. 224, 22 pp., illus.
(10) Hodson, W. E. H.
1932. narcissus pests. [Gt. Brit.] Min. Agr. and Fisheries Bul. 51, 40 pp., illus.
(11) ———
1935. control of insect pests of bulbs: a survey of recent work. Sci. Hort. [Wye, Kent] 3:192–196.
(12) McWhorter, F. P.
1932. narcissus "gray disease" is a transmissible mosaic. Florists' Exch. 79 (14): 11, illus.
(13) Slogteren, E. van.
1938. the transmission of virus diseases in daffodils. (Scientific Communication) Chron. Bot. 4: 205.
(14) Smith, K. M.
1933. recent advances in the study of plant viruses. 423 pp., illus. London.

ASYNAPTIC GOSSYPIUM PLANTS AND THEIR POLYPLOIDS[1]

By J. O. BEASLEY, *agronomist*, and META SUCHE BROWN, *cytologist*, *Texas Agricultural Experiment Station*

INTRODUCTION

In many hybrids between distantly related species chromosomes fail to pair during meiosis or pairing is incomplete (*4*, *5*)[2]. There are several known examples in which chromosomes fail to pair in nonhybrid individuals or in segregates from hybirds that usually give normal chromosome pairing (*1*, *2*, *4*, *5*, *9*, *10*). Doubling the chromosome number in hybrids in which most of the chromosomes fail to pair is usually followed by nearly normal chromosome pairing. From the work of Dobzhansky (*5*) doubling the chromosome number in nonhybrid individuals with reduced chromosome pairing, or in hybrids in which chromosomes have a reduced pairing as a result of gene combinations, would be expected to give no increase in the number of chromosomes paired.

Sterile plants were reported by Kearney (*8*) to segregate in the F_2 and F_3 of *Gossypium hirsutum* $\times$ *G. barbadense*, both of which are American cultivated tetraploid cottons with 26 pairs of chromosomes. Sterile plants from this cross have also been noted by Harland (*6*) and by others who have worked with the cross. Sterile plants were reported by Hutchinson and Gadkari (*7*) to segregate in a ratio of 3 fertile to 1 sterile in an Asiatic cotton with 13 pairs of chromosomes.

MEIOTIC CHROMOSOME BEHAVIOR IN ASYNAPTIC PLANTS AND THEIR POLYPLOIDS

In F_2 populations of *Gossypium hirsutum* $\times$ *G. barbadense*, plants were found that flowered but produced no seeds.[3] Anthers of some flowers dehisced, but examination with a hand lens indicated that the pollen was aborted. Apparently no functional embryo sacs or pollen were produced. Although some fruits reached nearly mature size, they had no seeds. Acetocarmine smears of pollen mother cells of F_1 plants show 26 pairs of chromosomes with no evidence of structural differences between the chromosomes (*3*), and fertile F_2 plants have the same meiotic chromosome behavior. In meiosis of the sterile plants less than half of the 52 chromosomes paired (fig. 1). The range in number of pairs was 2 to 15, and means of different sterile F_2 plants ranged from 6.3 to 11.6 (table 1). The chromosomes that paired usually had only one chiasma, and the univalents were scattered around the metaphase plate. At anaphase some bivalents separated sharply like normal bivalents with one chiasma, but some

[1] Received for publication, January 3, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 427.

[3] The first of the sterile plants used in this work were found in an F_3 progeny grown by Dr. Thomas Kerr, who also aided in examining the plants for sterility. Data were collected from other F_2 populations grown by T. R. Richmond; Dr. C. P. Swanson aided in the cytological work.

became attenuated. No fragments were found. At anaphase multipolar spindles frequently were present. In the second division the chromosomes formed regular spindles and apparently separated as

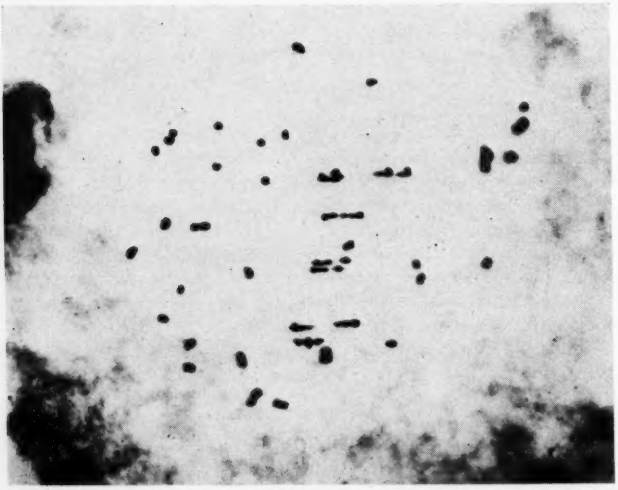

FIGURE 1.—First meiotic metaphase of sterile plant from F_2 progeny of *Gossypium hirsutum* × *G. barbadense* showing less than one-third of the chromosomes paired.

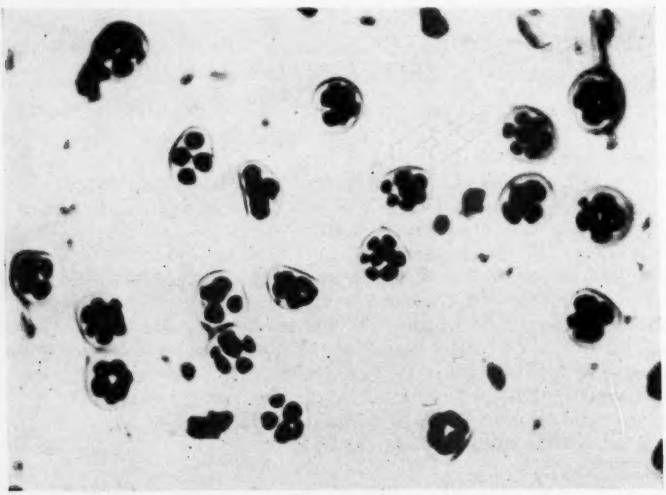

FIGURE 2.—Microsporocytes of sterile plant from F_2 progeny of *Gossypium hirsutum* × *G. barbadense* showing more than four microspores in "tetrads."

they do in normal plants. This meiotic chromosome behavior resulted in "tetrads" with 1 to 13 microspores (table 2) with extreme variability in size (fig. 2).

TABLE 1.—*Number of pairs of chromosomes in F_2 asynaptic plants from Gossypium hirsutum × G. barbadense*

Plant No[1]	Chromosome number	Pairs of chromosomes																															Number of nuclei	Mean
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1	4N					1	4	8	9	11	7	5	4			1			2		2	5	8	6	3	5	4	3	1	2		1		1	49	8.8
1 treated	8N																																		44	23.7
2	4N					1	7	10	7	6	6	4		1																					43	9.0
2 treated	8N																	1			1	1	1	4	2	3	5	3	2		1				24	24.7
3	4N							1	1	5	3	7	10	7	5	1					1	1	1	4	2	3	5	3	2						40	11.6
4	4N		1			3		3	3	2			2		1																			15	7.9
5	4N					1	2	6	1	3	1	2		1																					17	8.2
6	4N				3	2	3	12	4	3			1																						28	7.0
7	4N			2	6	3	12	4	8	3	2																								40	6.4
8[1]	4N				2	7	3	5	4	3			1																						25	6.7
9	4N				3		3	4	2	6	7	6	3																						34	8.8
10	4N		2	1		5	1	7	4		2	1		1																				24	6.8
11	4N		1	4	4	9	10	10	5	6	2	1																							52	6.3
12	4N						2	1	4	5	4	1	3	2																					22	9.5
13	4N			1		3	6	5	7	2	1																								28	7.3
14	4N					1	8	6	5	5	3			1	1																				30	7.9
15	4N							1	2	6	3	4	4	1	1	2																			27	10.9

[1] Plants 8–15 are from the same F_2 population.

TABLE 2.—*Number of microspores in "tetrads" of F_2 asynaptic plants from Gossypium hirsutum×G. barbadense*

Plant No.[1]	Chromosome number	Number of microspores																		Number of "tetrads"	Average
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1[1]	4N	1	1		12	18	42	23	35	10	8	4	2	1						157	6.9
1	4N		1	1	28	17	36	15	28	1	4									131	6.1
1	4N				16	12	54	58	36	12	3									191	6.7
1	4N			1	11	18	57	31	15	6										139	6.3
1 treated	8N		1		2	4	34	45	60	53	61	26	15	4	2	1				308	8.7
1 treated	8N				2	5	21	23	35	42	35	23	20	22	8	1	1			238	9.4
2	4N				11	19	46	37	47	32	15	9	5							221	8.1
2	4N				21	40	95	106	81	33	16	4	3	2						401	7.0
2 treated	8N					1		6	15	24	43	40	23	30	10	8	3		1	203	11.6
2 treated	8N		2		2	3	12	30	52	57	73	61	44	37	10	10	2	1		369	10.0
3	4N	13	62	40	714	183	162	37	10											1,221	4.4
3	4N	1	105	16	127	48	34	9	1											341	3.8
4	4N	1	24	13	34	21	34	48	19	5	2	2								203	5.5
5	4N				19	25	79	50	37	25	15	5								255	6.9
6	4N				4	8	29	32	38	33	30									174	7.8
7	4N			4	28	59	166	146	113	59	28	7	1							611	7.0
8[2]	4N		8	4	77	24	63	14	9		1									200	5.1
9	4N		3	4	30	29	45	19	6	2	2									140	5.5
10	4N			2	12	18	57	38	48	17	8									200	6.8
11	4N				1	2	8	5	26	21	25	24	16	9						137	9.6
11	4N			1	13	19	36	39	24	13	5	3								153	6.7
12	4N		3		275	81	112	17	5											493	4.7
13	4N				7	15	42	43	40	32	18	3								200	7.4
14	4N		10	21	95	28	20	4	1											179	4.2
15	4N		3	4	29	21	69	40	23	8	2		1							200	6.1

[1] Plants with same plant number and chromosome number represent collections made from same plant on different dates.
[2] Plants 8–15 are from same F_2 population

The pachytene chromosomes of *Gossypium* are unfavorable for study, but the observations that were made indicated that most of the chromosomes were paired at pachytene, as reported in asynaptic Zea (*2*) and Pisum (*9*).

The chromosome number of two asynaptic plants was doubled by colchicine treatment of grafts made from these plants. In meiosis 46 percent of the chromosomes of the treated plants paired as compared with 34 percent in the original asynaptic plants. Since environment is known to influence chromosome pairing and the material was collected at different times, the data do not justify the conclusion that doubling the chromosome number increased the amount of pairing. The range in the number of paired chromosomes in the asynaptic plants with double the original number of chromosomes was 15 to 33 (table 1 and fig. 3). The number of microspores in the "tetrads" reached as high as 18 (table 2). A few trivalents and quadrivalents were found in asynaptic plants with double the original number of chromosomes.

GENETICS OF ASYNAPTIC GOSSYPIUM PLANTS

An F_2 progeny of *Gossypium hirsutum* (Stoneville 5)×*G. barbadense* (Seabrook) gave 125 fertile plants and 8 asynatic plants (table 3). A cross between an upland variety, Coker 100, and a sea-island strain, which was probably Bleakhall, gave 49 fertile and 5 sterile plants. A cross reported by Kearney (*8*) of *G. hirsutum* (Holden)×*G. barbadense* (Pima) gave 200 fertile and 15 sterile plants. Kearney's description

leaves no doubt that the sterile plants he found were the asynaptic type. Of 22 F_3 progenies which he obtained from fertile F_2 plants, 9 contained from 3 to 38 percent of completely sterile plants. Since the F_2 ratio shows the asynaptic plants to be the result of two genes, both of which must be homozygous recessive, some F_3 progenies would be expected to segregate 3 fertile to 1 sterile, and others would be expected to segregate 15 fertile to 1 sterile. In the F_3 53.3 percent or 12 of 22 progenies would be expected to have sterile segregates. The range of 3 to 38 percent of sterile plants in F_3 progenies would be expected in small F_3 populations.

No asynaptic plants were found in an F_2 population of over 500 of *G. hirsutum* (Half and Half)×*G. barbadense* (Seabrook), in a population of over 100 of *G. hirsutum* (Half and Half)×*G. barbadense* (Pima), or in a population of about 125 of *G. hirsutum* (Acala 8)×*G. barbadense* (Seabrook).

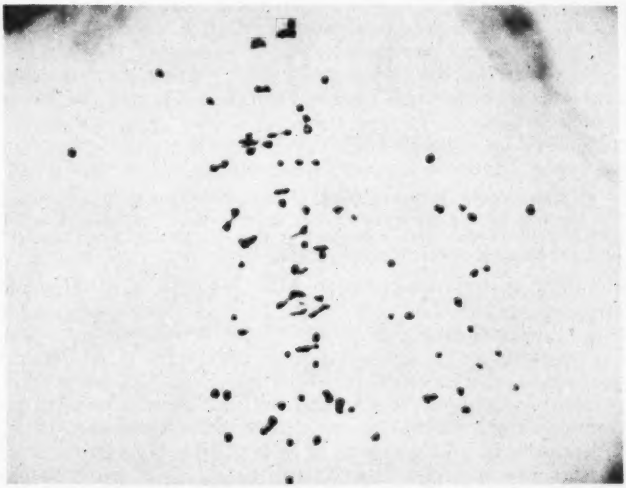

FIGURE 3.—First meiotic metaphase of sterile plant with double the original number of chromosomes from F_2 progeny of *Gossypium hirsutum*×*G. barbadense* showing that doubling the chromosome number failed to restore normal pairing of the chromosomes.

DISCUSSION

The F_2 ratio of 15 to 1 shows that the asynaptic *Gossypium* plants are the result of two pairs of recessive genes, one of which was shown to be homozygous in certain strains from three varieties of *G. hirsutum* and the other in two varieties of *G. barbadense*. Prof. G. W. Beadle has suggested to the authors that the situation might possibly be due to the same gene occurring homozygous in each of the two sets of chromosomes. Strains of two varieties of *G. hirsutum* (Half and Half and Acala 8) did not have the recessive gene. The mean difference in the number of bivalents and number of microspores in the "tetrads" of different asynaptic segregates collected the same day from the same population is evidence that modifying factors are present. Similar modifying factors were found by Beadle (*1*) in asynaptic *Zea* plants.

TABLE 3.—*Normal and asynaptic plants in F_2 populations of Gossypium hirsutum × G. barbadense*

Cross	Normal	Asynaptic	$\frac{D}{P.E.}$
G. hirsutum (Stoneville 5)×*G. barbadense* (Seabrook)	125	8	<1.0
G. hirsutum (Coker 100)×*G. barbadense* (Bleakhall?)	49	5	1.3
G. hirsutum (Holden)×*G. barbadense* (Pima) [1]	200	15	<1.0
Total	374	28	<1.0

[1] Data from Keraney (*8*).

The percentage of the chromosomes pairing in the original asynaptic plants and in the plants with double the original number of chromosomes was approximately equal. The chromosomes failed to pair in the original F_2 plants because of a gene combination. In doubling the chromosome number, the physiological effect of the genes in question was not changed, so pairing conditions remained the same. This fact had been noted by Dobzhansky (*5*) from studies of occasional polyploid cells in F_1 hybrids between the A and B races of *Drosophila pseudoobscura*.

A statement by Dobzhansky concerning a sterile hybrid and its fertile polyploid finds no support in *Gossypium*. He states (*5, p. 326*):

> * * * One cannot, however, exclude the possibility that the chromosome pairing in the diploid is suppressed by the hybrid genetic constitution, but that the doubling of the chromosome complement entails a physiological change which removes the hindrance to bivalent formation.

If doubling the chromosome number resulted in a physiological reaction allowing the chromosomes to pair, it is probable that many of the chromosomes would form multivalents which would result in unstable polyploids. In all examples of polyploids in which chromosome pairing became normal, or nearly so, following the doubling of the chromosome number in a hybrid with no pairing or with pairing much reduced from normal, it is believed that the failure of pairing in the initial hybrid was the result of structural and genetic differences between the chromosomes, rather than the action of genes to prevent chromosome pairing. Evidence has been given (*3*) that structural differences exist between all the chromosomes in certain *Gossypium* hybrids that yielded highly fertile polyploids.

In asynaptic *Zea* plants Beadle (*2*) found an occasional attenuated bivalent and in some cells fragments. Koller (*9*) found attenuated bivalents in asynaptic *Pisum*, but he had evidence to show that the plant was heterozygous for an inversion, for no bridges were found in other asynaptic *Pisum* plants. In the asynaptic *Gossypium* no evidence was found that the attenuated chromosomes are the result of structural differences between chromosomes. It may be possible that the upset in meiosis that resulted in asynapsis could also at times cause attenuated bivalents. That these are not identical with bridges due to structural differences is evidenced by normal pairing in the F_1. Furthermore, doubling of the chromosome number in hybrids where pairing was absent or incomplete because of structural differences resulted in nearly normal pairing (*3, 5*), whereas doubling the number

of chromosomes in asynaptic plants produced little or no change in percentage of paired chromosomes.

It has been suggested that regular formation of bivalents, rather than multivalents, and normal fertility might be produced in autopolyploids by inducing mutations which reduce chiasma frequency. That this idea has no promise, at least in *Gossypium*, is shown by the autopolyploids from the asynaptic plants, for with only 46 percent of the chromosomes paired some trivalents and quadrivalents were present.

SUMMARY

In an F_2 progeny of *Gossypium hirsutum* × *G. barbadense* (American upland × sea-island cotton), sterile asynaptic plants were found in a ratio of 15 fertile to 1 sterile. At first metaphase the fertile plants had the normal 26 pairs of chromosomes while the asynaptic plants averaged 6 to 12 pairs of chromosomes. Doubling the chromosome number in the sterile asynaptic plants failed to restore normal chromosome pairing and fertility.

LITERATURE CITED

(1) Beadle, G. W.
1930. Genetical and cytological studies of Mendelian asynapsis in zea mays. N. Y. (Cornell) Agr. Expt. Sta. Mem. 129, 23 pp., illus.

(2) ———
1933. Further studies of asynaptic maize. Cytologia 4: 269–287, illus.

(3) Beasley, J. O.
1942. Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyploids of gossypium. Genetics 27: 25–54, illus.

(4) Darlington, C. D.
1937. Recent advances in cytology. Ed. 2, 671 pp., illus. Philadelphia.

(5) Dobzhansky, T.
1941. Genetics and the origin of species. Ed. 2, 446 pp., illus. New York.

(6) Harland, S. C.
1939. The genetics of cotton. 193 pp., illus. London.

(7) Hutchinson, J. B., and Gadkari, P. D.
1935. A note on the inheritance of sterility in cotton. Indian Jour. Agr. Sci. 5: 619–623.

(8) Kearney, T. H.
1923. Segregation and correlation of characters in an upland-egyptian cotton hybrid. U. S. Dept. Agr. Dept. Bul. 1164, 58 pp., illus.

(9) Koller, P. C.
1938. Asynapsis in pisum sativum. Jour. Genet. 36: [275]–306, illus.

(10) Smith, L.
1936. Cytogenetic studies in triticum monococcum l. and t. aegilopoides bal. Mo. Agr. Expt. Sta. Res. Bul. 248, 38 pp., illus.

THE INFLUENCE OF FEEDING LOW-NITROGEN RATIONS ON THE RELIABILITY OF BIOLOGICAL VALUES[1]

By J. I. MILLER, *assistant professor in animal husbandry*, and F. B. MORRISON, *animal husbandman, and animal nutritionist, and head, Department of Animal Husbandry, New York (Cornell) Agricultural Experiment Station*[2]

INTRODUCTION

For the past several years nitrogen balance experiments with lambs have been conducted at this station to study the quality of protein furnished by common feedstuffs (*10*, *11*, *20*, *21*).[3] The nutritive value of the protein has been expressed as the percentage of total nitrogen stored, the percentage of digested nitrogen stored, and the biological value. In the later work the biological values reported are termed "estimated biological values," since average values for endogenous and metabolic nitrogen as determined in earlier experiments with lambs were used in the calculations.

The usual method of determining biological values of protein involves feeding a nitrogen-free or low-nitrogen ration during the experiment in order to measure the endogenous nitrogen losses in the urine and the metabolic nitrogen losses in the feces (*12*). Because of the difficulty in inducing experimental animals to consume sufficient amounts of either a nitrogen-free ration or a ration very low in nitrogen (*8*), certain investigators have added small amounts of some protein of high quality, such as egg protein. According to data reported by Mitchell and Carmen (*14*), the nitrogen excretions of rats fed a ration containing 0.6 to 0.75 percent of whole-egg nitrogen (3.75 to 4.69 percent protein) were practically the same as when they were fed a ration nearly nitrogen-free.

The time required by animals fed a low-nitrogen or nitrogen-free ration to reach an endogenous level of nitrogen losses is not definitely established. Smuts reports the time required by various animals to reach the endogenous level to be as follows: Mice, 5 days; guinea pigs, 8 days; rabbits, 15 days; and pigs, 20 days (*17*). Mitchell (*12*) and Chick and Roscoe (*5*), as well as others, have commonly used periods of 3 to 5 days for measuring the endogenous losses for rats. McCollum (*6*) and Mitchell and Hamilton (*15*) have used 10 to 15 days for swine. Turk et al. (*20*) and Sotola (*19*) have used a 10-day standardizing period for sheep. Martin and Robison report that for human subjects 5 to 7 days are required (*7*).

Ashworth and Brody (*1*, *2*, *3*), found that the minimum urinary nitrogen levels for rats were obtained at any time between 4 and 28 days on a nitrogen-free diet. Rats which had been previously fed a low-protein ration required less time to reach the endogenous level than rats previously fed a high-protein ration. Chick, Hutchinson, and Jackson (*4*) report that the excretion of endogenous nitrogen in the

[1] Received for publication January 19, 1942.
[2] The authors acknowledge their indebtedness to Dr. L. A. Maynard, professor of animal nutrition, for helpful suggestions relative to this study and to Paul Johnson for doing much of the chemical analysis.
[3] Italic numbers in parentheses refer to Literature Cited, p. 450.

urine tends to diminish with the length of time that a rat is fed a diet devoid of nitrogen, although a period of 2 to 3 days is long enough to include the initial rapid fall in nitrogen excretion. They consider that a collection period of 4 days following a preliminary period of 2 to 3 days gives a fairly good estimate of the endogenous nitrogen in the urine, although the value may be regarded as a somewhat arbitrary quantity. Mitchell et al. (*13*) concluded that the level of endogenous nitrogen excretion as measured by their procedure possesses a definite biological significance and that the biological values determined possess an absolute as well as a relative significance.

In work at the Cornell station (*9, 21*), data have been obtained which might indicate that lambs store a higher percentage of the protein in an experimental ration following the feeding of a low-nitrogen ration. Considerable time may be required by lambs to reach a more or less stable level of protein utilization after such feeding and a 10-day preliminary period may not be of sufficient length.

Various problems have arisen regarding the accurate determination of the metabolic nitrogen fraction of the feces (*16*). The most common manner of expressing the metabolic nitrogen loss is in terms of dry-matter intake. Although body weight and possibly other factors may have some influence, dry-matter intake seems to be the predominant factor. However, it has been noted that when the experimental animals consumed but small amounts of the low-nitrogen rations, the amount of metabolic nitrogen excreted per unit of dry-matter intake was higher.

OBJECT OF EXPERIMENTS

The series of experiments with lambs reported in this paper were planned to obtain information on the following points:

(1) The length of time required by lambs fed a low-nitrogen ration to reach their endogenous nitrogen level.

(2) The effect on feed consumption, on losses in body weight, and on nitrogen excretion of adding small amounts of dried skim milk or of corn-gluten meal to low-nitrogen rations.

(3) The time required by lambs to reach a stable level of nitrogen utilization when fed rations containing approximately 10 percent or more protein following the feeding of a low-nitrogen ration.

(4) The influence of a low-nitrogen ration on the accuracy of biological values as heretofore obtained with lambs as a measure of quality of protein.

EXPERIMENT 1

PROCEDURE

Continuous nitrogen balance data were obtained on two grade wether lambs of mutton type by the use of metabolism cages. These cages, as well as the methods used in conducting the balance experiment, have been described previously (*20*). The urine and feces were collected at 2-day intervals and analyzed for nitrogen by the standard Kjeldahl procedure. Previous to the experiment the lambs had been fed a ration containing approximately 10 percent protein. each lamb was fed the three different rations shown in table 1.

TABLE 1.—*Percentage composition and average protein content of rations used in experiment 1*

Ingredients	Low-nitrogen ration	High-nitrogen ration	Nitrogen-poor ration
	Percent	*Percent*	*Percent*
Cellulose	10.5	9.0	9.0
Starch	28.0	11.0	24.2
Sugar	28.0	10.7	24.2
Wheat straw	25.0	25.0	25.0
Corn oil	4.5	3.0	3.0
Minerals [1]	4.0	3.0	3.0
Dried skim milk		11.6	11.6
Linseed meal		26.7	
Total	100.0	100.0	100.0
Average protein content (N×6.25)	1.05	13.16	4.49

[1] The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

Changes between rations were made abruptly by substituting one ration for another at a morning feeding, and collection of excreta was begun the same day. The first ration, which was fed for a period of 34 days, was the low-nitrogen basal ration for lambs developed at this station. The second ration, a high-nitrogen ration, was fed for 20 days following the feeding of the low-nitrogen ration. The purpose of feeding this ration was to bring the lambs back to a good state of nutrition, as well as to study the utilization of nitrogen immediately following a period of low-nitrogen intake.

Finally, a nitrogen-poor ration was fed for 40 days. This ration differed from the low-nitrogen ration mainly in containing enough dried skim milk to furnish approximately an average of 4 percent of protein and to bring the protein content of the ration to an average of 4.49 percent. Dried skim milk was selected as a feed which should furnish protein of as high quality as any feed which would be feasible to use in rations for lambs. If lambs respond in a manner similar to that reported for rats by Mitchell and Carmen (*14*), the losses of nitrogen of lambs fed this amount of protein in the ration should have been essentially the same as when they were receiving a nitrogen-free ration. The high-nitrogen ration, fed in the second period, was the nitrogen-poor ration plus sufficient linseed meal to furnish approximately 10 percent protein.

RESULTS

The results of experiment 1 are presented in table 2 and in figures 1, 2, 3, and 4. The curves presented in these and later figures are calculated logarithmic curves. The dots represent the actual determinations for each lamb.

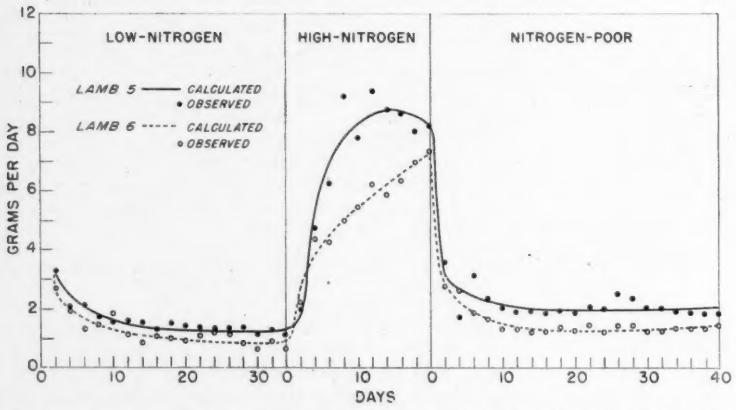

FIGURE 1.—Average daily losses of nitrogen in the urine of lambs fed a low-nitrogen ration, a high-nitrogen ration, and a nitrogen-poor ration.

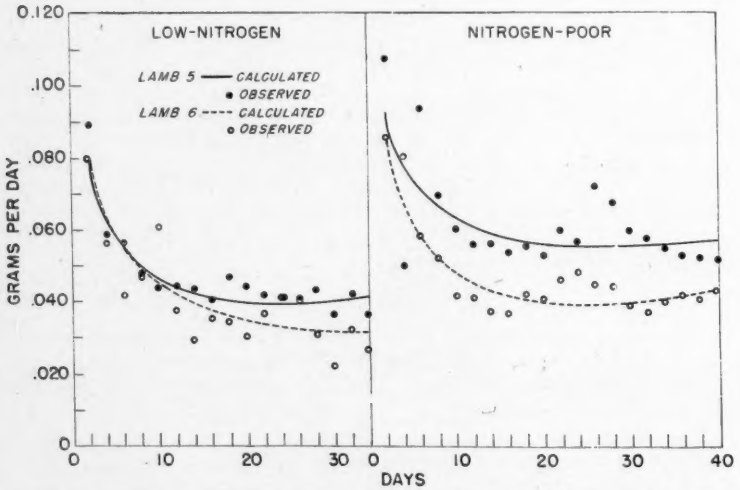

FIGURE 2.—Average daily losses of nitrogen per kilogram of body weight of the lambs when on the low-nitrogen ration and on the nitrogen-poor ration.

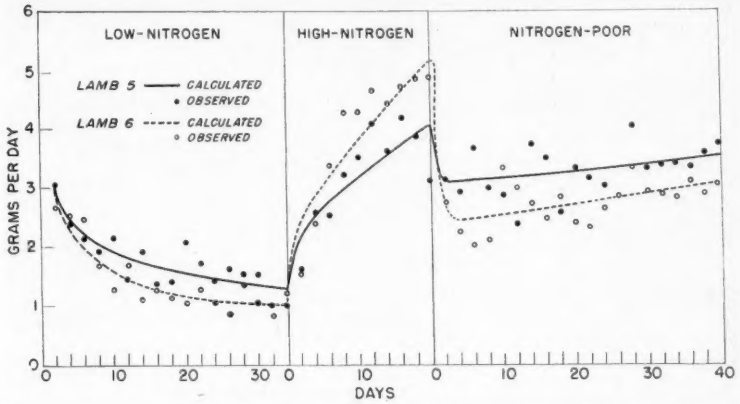

FIGURE 3.—Average daily losses of nitrogen in the feces of lambs fed a low-nitrogen ration, a high-nitrogen ration, and a nitrogen-poor ration.

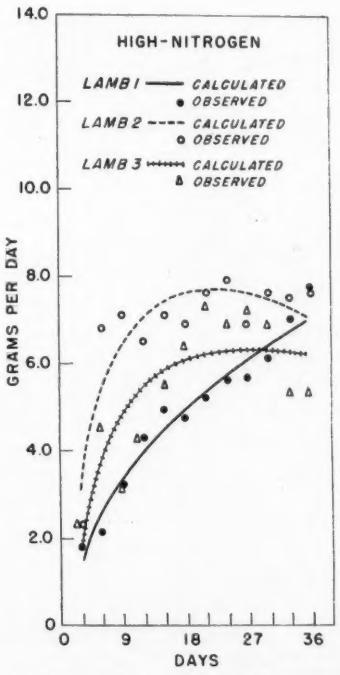

FIGURE 4.—Average daily losses of nitrogen in the feces per 100 gm. of dry-matter intake of lambs fed a low-nitrogen ration and a nitrogen-poor ration.

TABLE 2.—*Nitrogen-balance data of experiment 1 by 2-day periods*

LOW-NITROGEN RATION

Days on ration	Weight		Feed intake		Dry matter intake		Nitrogen intake		Nitrogen in urine		Nitrogen in feces	
	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6
	Kg.	*Kg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
2	36.4	33.3	1,200	1,200	1,119.0	1,119.0	2.10	2.10	6.52	5.32	6.10	5.33
4	36.2	32.4	1,200	900	1,119.0	839.2	2.10	1.58	4.06	3.78	4.72	5.00
6	35.9	31.8	1,200	1,200	1,119.0	1,119.0	2.10	2.10	4.06	2.65	4.29	4.93
8	35.7	31.1	1,200	400	1,119.0	373.0	2.10	.70	3.43	2.88	3.84	3.35
10	35.4	30.6	1,200	700	1,119.0	652.8	2.10	1.22	3.13	3.69	4.30	2.52
12	34.8	30.2	500	900	465.1	837.2	.84	1.52	3.10	2.26	2.90	3.37
14	34.3	29.5	850	650	790.7	604.6	1.43	1.10	2.99	1.73	3.83	2.20
16	32.1	29.0	600	700	558.1	651.1	1.01	1.18	2.59	2.06	2.73	2.59
18	31.7	28.8	700	600	651.1	558.1	1.18	1.01	2.98	2.00	2.80	2.25
20	31.8	28.6	1,000	525	930.2	488.4	1.68	.88	2.82	1.75	4.10	2.04
22	32.2	29.1	900	600	837.2	558.1	1.52	1.01	2.70	2.15	3.41	2.57
24	31.5	28.5	600	250	556.7	232.0	.97	.40	2.62	2.42	2.85	2.10
26	31.4	28.5	800	650	742.3	603.1	1.30	1.15	2.59	2.28	3.27	1.70
28	30.8	27.8	800	700	742.3	649.5	1.30	1.13	2.69	1.73	3.08	2.72
30	30.6	27.7	800	400	742.3	371.2	1.30	.65	2.23	1.26	3.04	2.06
32	30.1	27.2	250	325	232.0	301.6	.40	.53	2.54	1.75	2.01	1.67
34	29.6	27.1	600	567	556.7	526.1	.97	.92	2.15	1.45	1.97	2.43

HIGH-NITROGEN RATION

2	29.8	27.4	750	750	698.8	698.8	15.79	15.79	3.87	4.43	3.20	3.06
4	30.5	28.1	1,100	1,100	1,025.0	1,025.0	23.16	23.16	9.28	8.66	5.11	5.26
6	30.4	28.7	1,225	1,225	1,141.5	1,141.5	25.79	25.79	12.36	8.50	5.02	6.75
8	31.3	29.4	1,325	1,325	1,234.6	1,234.6	27.89	27.89	18.34	9.90	6.41	8.54
10	31.5	30.0	1,550	1,550	1,444.3	1,444.3	32.63	32.63	15.54	10.98	6.99	8.52
12	31.8	30.7	1,600	1,600	1,490.9	1,490.9	33.68	33.68	18.74	12.36	8.17	9.30
14	31.3	30.1	1,600	1,600	1,490.9	1,490.9	33.68	33.68	17.40	11.68	7.19	7.86
16	32.8	31.6	1,600	1,600	1,490.9	1,490.9	33.68	33.68	17.22	12.64	8.33	8.40
18	32.7	32.0	1,600	1,600	1,490.9	1,490.9	33.68	33.68	15.97	13.94	7.68	9.67
20	32.5	32.1	1,600	1,600	1,490.9	1,490.9	33.68	33.68	16.38	14.62	6.22	9.74

NITROGEN-POOR RATION

2	33.1	32.3	1,600	1,200	1,493.8	1,120.3	11.62	8.71	7.14	5.52	6.23	5.42
4	34.0	32.6	1,600	500	1,493.8	466.8	11.62	3.63	3.40	5.25	5.82	4.46
6	33.2	31.7	1,600	900	1,493.8	840.2	11.62	6.53	6.24	3.71	7.25	4.01
8	33.5	31.2	1,600	1,100	1,493.8	1,027.0	11.62	7.99	4.70	3.26	5.93	4.22
10	34.0	31.3	1,600	1,450	1,493.8	1,353.7	11.62	10.53	4.11	2.63	5.67	6.63
12	34.2	31.5	1,600	1,300	1,493.8	1,213.7	11.62	9.44	3.83	2.61	6.72	5.98
14	34.3	31.8	1,600	1,250	1,493.8	1,167.0	11.62	9.08	3.88	2.37	7.44	5.41
16	34.1	32.0	1,600	1,250	1,493.8	1,167.0	11.62	9.08	3.67	2.38	6.98	4.91
18	34.1	31.6	1,600	500	1,493.8	466.8	11.62	3.63	3.77	2.68	5.13	5.65
20	34.5	30.8	1,600	850	1,477.8	785.1	11.41	6.06	3.66	2.54	6.62	4.77
22	34.2	31.0	1,600	1,050	1,477.8	969.8	11.41	7.49	4.14	2.89	6.28	4.63
24	34.0	31.3	1,600	1,400	1,477.8	1,293.0	11.41	9.98	3.88	2.42	6.01	5.22
26	35.0	31.6	1,600	1,400	1,477.8	1,293.0	11.41	9.98	5.06	2.84	5.59	5.66
28	34.4	32.0	1,600	1,600	1,477.8	1,477.8	11.41	11.41	4.68	2.86	8.05	7.16
30	34.6	32.4	1,600	1,350	1,482.6	1,250.9	11.36	9.58	4.14	2.54	6.63	5.81
32	34.5	32.4	1,600	1,250	1,482.6	1,158.2	11.36	8.88	3.99	2.40	6.74	5.73
34	34.3	32.1	1,600	1,200	1,482.6	1,111.9	11.36	8.52	3.77	2.60	6.73	5.59
36	34.2	31.3	1,600	1,400	1,482.6	1,297.2	11.36	9.94	3.66	2.64	6.63	6.12
38	35.0	32.2	1,600	1,400	1,482.6	1,297.2	11.36	9.94	3.66	2.64	7.12	5.76
40	34.7	32.1	1,600	1,350	1,482.6	1,250.9	11.36	9.58	3.62	2.78	7.40	6.09

FEED INTAKE

Each lamb was fed 600 gm. per day of the low-nitrogen ration at the start with the hope that at this rather low level of intake all the feed would be consumed. However, after a short time, neither lamb would consume that amount of feed. Lamb 5 tended to have a stronger appetite than lamb 6 throughout the period, although this lamb refused to consume 600 gm. of the low-nitrogen ration after the tenth day. After each lamb had refused to eat as much of the ration as planned, the ration was fed according to appetite.

The appetite of each lamb improved markedly when the high-nitrogen ration was fed. Both lambs made a regular increase in feed consumption until each consumed 800 gm. per day after the tenth day. This level of feeding was continued during the remainder of the period on this ration.

Lamb 5 continued to eat 800 gm. per day when fed the nitrogen-poor ration and his appetite remained good. The appetite of lamb 6 was somewhat irregular on this ration. He ate less than when fed the high-nitrogen ration, but considerably more than when fed the low-nitrogen ration.

Body Weight

Both lambs lost weight steadily during the period in which the low-nitrogen ration was fed. The losses were greater during the intervals of low-feed intake. However, at no time did the lambs maintain their weight when fed the low-nitrogen ration. Each lamb started to gain in weight immediately upon changing to the high-nitrogen ration, and continued making good gains throughout the period.

Lamb 5 continued to make slight gains in weight when fed the nitrogen-poor ration. This small gain amounted to approximately 1.5 kg. for the 40-day period. Probably because of lower feed consumption, lamb 6 just maintained his weight when fed this ration.

Urinary Nitrogen Losses

The urinary nitrogen losses declined abruptly during the first 10 days the lambs were fed the low-nitrogen ration, but after the first 10 or 12 days, the decline was slight (fig. 1).

The fact that the lambs excreted the largest daily amount of urinary nitrogen during the first 10 days of feeding may be explained largely by the residual effect of the former ration, which contained approximately 10 percent protein. The small decline in rate of excretion of urinary nitrogen following this initial drop may be due, in part, to the fact that the lambs were gradually losing in body weight. Also, the lambs may have become gradually more efficient in the utilization of the nitrogen until they reached a so-called endogenous level.

As would be expected, the urinary nitrogen losses increased when the lambs were fed the high-nitrogen ration. However, the amount excreted was relatively small at the start, showing that nitrogen was being retained in large amounts, with considerable of it undoubtedly remaining in the digestive tract. After approximately the tenth day, the nitrogen excretion in the urine did not change greatly. However, as indicated in figure 1, there was no positive evidence that the lambs had reached a fairly constant level of nitrogen excretion in the urine during this 20-day period.

The urinary nitrogen dropped immediately when the lambs were fed the nitrogen-poor ration. However, as shown in figure 1, it did not reach such low levels when the lambs were fed this ration containing 4.49 percent protein chiefly from dried skim milk as when they were fed a low-nitrogen ration containing 1.05 percent protein.

It would therefore be obviously incorrect to consider that the urinary nitrogen excretion on the nitrogen-poor ration represented solely endogenous nitrogen excretion. Without doubt some of the nitrogen excreted during this period was of feed origin.

As shown in figure 2, the losses of urinary nitrogen per kilogram of body weight follow the same trend as that of the total urinary nitrogen excretion. The greatest drop occurred during the first 10 days. After this drop there was no positive evidence that the values for endogenous nitrogen per kilogram of weight became progressively lower with time.

Fecal Nitrogen Losses

Fecal nitrogen losses were also greater when the low-nitrogen ration was first fed than after the first 6 or 8 days. This initial high excretion of nitrogen in the feces was undoubtedly due mainly to the previous higher level of feeding and higher nitrogen content of the ration. After this short time, the fecal nitrogen excretion was definitely correlated with the total feed intake on the low-nitrogen ration.

Another lag in fecal nitrogen excretion was observed when the lambs were first fed the high-nitrogen ration. As shown in figure 3, the nitrogen losses were appreciably smaller until after approximately the tenth day. Following this time the fecal nitrogen losses appeared to be mainly correlated with the feed or nitrogen intake.

The fecal nitrogen dropped to a lower level when the lambs were fed the nitrogen-poor ration instead of the high-nitrogen ration. However, considerably more nitrogen was excreted than when the low-nitrogen ration was fed. This level of nitrogen excretion remained more or less constant for each lamb throughout the feeding of the nitrogen-poor ration.

The values for metabolic nitrogen calculated by 2-day periods show a strong correlation between feed intake and fecal nitrogen losses (fig. 4). If the first few periods are omitted, the fecal nitrogen losses of the lambs on both the low-nitrogen and the nitrogen-poor rations appear to be governed by the level of feed intake rather than by the nitrogen content or by the length of time the lambs were fed the rations.

The nitrogen in the high-nitrogen ration was utilized with high efficiency. During the last 10 days of the high-nitrogen period of feeding, lamb 5 retained on an average 26.8 percent of the total nitrogen in the ration while lamb 6 retained an average of 34.6 percent. The percentage of total nitrogen retained by the lambs during the first 10 days of this period was even greater.

In a previous experiment at this station (*9*), in which there were no preliminary low-nitrogen periods, lambs fed a ration containing approximately 10 percent of protein furnished almost entirely by dried skim milk stored 19.8 percent of the nitrogen. Lambs fed a similar ration except that it contained linseed meal as the source of protein stored 22.7 percent of the total nitrogen. These lower values for nitrogen storage were obtained even though less protein was fed than in the experiment reported in this paper. Therefore these high values for nitrogen utilization were probably due largely to the influence of the previous period of low-nitrogen feeding.

EXPERIMENT 2

PROCEDURE

In the second experiment, continuous nitrogen balance data were obtained on four lambs. The methods used were similar to those

employed in experiment 1, except that the collection of the excreta was made at 3-day intervals instead of 2-day intervals, in order to lessen the cost.

Each of the four lambs was fed a ration containing slightly more than 10 percent protein during an initial period of 20 days. Lambs 1 and 2 were fed a ration in which most of the protein was furnished by soybean oil meal, while lambs 3 and 4 received a similar ration except that it contained corn-gluten meal. Nitrogen balances were determined on each lamb during the last 9 days of the period.

Following the above initial experimental period, each lamb was fed a low-nitrogen ration containing approximately 2.75 percent protein. The change in ration was made abruptly at a morning feeding and the nitrogen balances were continued without interruption.

The rations used in this period were the same as the low-nitrogen basal ration used during the first phase of experiment 1, except that some of the starch and sugar was replaced with dried skim milk or corn-gluten meal. These protein-rich feeds furnished approximately 1.2 percent protein in the rations, or nearly one-half the total protein, with wheat straw furnishing the remainder. Lambs 1 and 3 were fed the dried skim milk ration and lambs 2 and 4 were fed the corn-gluten meal ration.

The original plan was to obtain nitrogen balance data on each lamb during an experimental period of 51 days without change from the low-nitrogen ration. Unfortunately, this plan was actually followed on only one lamb, as explained in the discussion of results.

In the last phase of this experiment, each lamb was again fed the same ration as at the start. Collections were made and nitrogen balances were obtained on each lamb through a period of 39 days. The rations fed during experiment 2 are given in table 3.

TABLE 3.—*Percentage composition, and average protein content of rations used in experiment 2*

Ingredients	High-nitrogen rations		Low-nitrogen rations	
	Soybean oil meal	Corn-gluten meal	Dried skim milk	Corn-gluten meal
	Percent	*Percent*	*Percent*	*Percent*
Cellulose	9.0	9.0	10.5	10.5
Starch	17.78	18.31	26.32	26.83
Sugar	17.77	18.30	26.32	26.84
Wheat straw	25.0	25.0	25.0	25.0
Corn oil	3.0	3.0	4.0	4.0
Minerals [1]	3.0	3.0	4.0	4.0
Soybean oil meal	24.45			
Corn-gluten meal		23.39		2.73
Dried skim milk			3.86	
Total	100.00	100.00	100.00	100.00
Average protein content (N × 6.25)	11.03	10.76	2.73	2.75

[1] The mineral mixture was made up of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

RESULTS

The results of experiment 2 are shown in table 4 and in figures 5 and 6.

TABLE 4.—*Nitrogen balance data of experiment 2 by 3-day periods*

HIGH-NITROGEN RATION

Days on ration	Weight of lamb				Feed intake				Dry-matter intake				Nitrogen intake				Nitrogen in urine				Nitrogen in feces			
	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4
	Kg.	*Kg.*	*Kg.*	*Kg.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
3	31.2	33.6	34.2	34.2	2,400	2,400	2,400	2,400	2,215.4	2,215.4	2,229.6	2,229.6	43.68	43.68	44.40	44.40	14.50	20.01	17.98	27.60	15.47	14.37	12.99	11.71
6	32.2	33.4	35.1	33.7	2,400	2,400	2,400	2,400	2,215.2	2,215.2	2,229.6	2,229.6	43.64	43.64	44.40	44.40	18.43	23.80	28.22	26.37	14.31	14.68	15.90	18.43
9	32.0	33.9	34.3	33.9	2,400	2,400	2,400	2,400	2,215.2	2,215.2	2,219.0	2,219.0	43.64	43.64	44.16	44.16	27.26	20.28	21.81	29.42	15.64	17.11	12.32	14.30

LOW-NITROGEN RATION

3	31.2	33.6	34.0	34.4	2,000	2,400	2,200	2,400	1,852.8	2,238.2	2,038.1	2,238.2	8.20	9.12	9.02	9.12	10.94	8.99	9.05	9.78	13.59	17.76	12.75	13.08
6	30.6	33.7	34.1	34.2	2,150	2,400	1,800	972	1,991.8	2,238.2	1,667.5	906.5	8.82	9.12	7.38	3.69	4.13	4.37	4.55	7.26	10.62	13.60	10.08	7.37
9	30.6	33.9	34.1	32.5	1,900	1,924	2,100	1,500	1,760.2	1,794.3	1,945.4	1,398.9	7.79	7.31	8.61	5.70	4.10	5.13	4.02	5.94	8.27	10.74	5.84	6.57
12	30.4	32.7	34.6	32.7	1,800	600	2,000	1,800	1,667.5	559.6	1,852.8	1,678.7	7.38	2.28	8.20	6.84	3.64	8.36	3.55	3.39	6.74	6.01	9.33	7.51
15	30.4	30.8	34.4	32.3	1,000	1,700	1,800	1,400	940.7	1,584.4	1,693.3	1,305.6	4.14	6.46	7.45	5.32	5.88	6.90	4.15	3.18	6.50	7.49	8.28	6.98
18	28.9	31.8	34.4	31.7	1,200	1,800	1,800	1,100	1,128.8	1,687.3	1,693.3	1,031.1	4.97	30.78	7.45	18.81	4.32	4.85	4.12	3.89	7.10	9.84	8.76	6.65
21	28.4	31.7	34.2	31.0	1,300	1,800	1,800	1,500	1,222.9	1,687.3	1,693.3	1,496.1	5.38	30.78	7.45	25.65	3.38	16.04	3.80	8.26	7.55	10.49	8.28	6.09
24	28.0	30.9	34.4	31.9	700	1,809	1,800	1,800	658.5	1,687.3	1,693.3	1,687.3	2.90	30.78	7.45	30.78	6.25	20.78	3.75	13.12	5.35	12.54	8.09	10.21
27	27.3	30.7	33.6	32.2	300	1,800	1,800	1,800	282.2	1,687.3	1,693.3	1,687.3	1.24	30.78	7.45	30.78	7.66	14.85	3.56	16.51	3.48	11.18	8.06	11.05
30	25.8	31.6	33.5	32.7	250	1,800	1,800	1,800	235.2	1,687.3	1,693.3	1,687.3	1.04	30.78	7.45	30.78	9.08	16.31	3.47	21.71	2.53	8.28	8.09	9.62
33	25.1	31.8	33.5	33.0	204	1,800	1,800	1,800	191.9	1,687.3	1,693.3	1,687.3	.84	30.78	7.45	30.78	10.90	19.34	4.08	15.13	3.12	8.33	7.94	8.34
36	23.8	31.6	33.3	33.2	8	1,800	1,800	1,800	7.5	1,685.2	1,693.3	1,685.2	33	6.91	7.45	6.91	10.27	8.96	3.77	11.19	9.93	8.03	7.82	10.50
39	.....	33.0	32.9	33.5	.....	1,800	1,800	1,800	.....	1,685.2	1,693.3	1,685.2	.....	6.91	7.45	6.91	.....	4.74	3.35	3.77	.....	10.20	9.06	8.87
42	.....	33.0	31.8	33.1	.....	1,809	900	1,400	.....	1,685.2	836.6	1,310.7	.....	6.91	4.05	5.37	.....	3.73	4.01	3.52	.....	7.66	5.00	12.36
45	.....	32.1	31.4	32.8	.....	1,800	1,200	1,800	.....	1,685.2	1,115.5	1,685.2	.....	6.91	5.40	6.91	.....	3.75	3.83	4.01	.....	7.72	5.44	7.00
48	.....	33.4	31.6	32.9	.....	1,800	1,300	1,800	.....	1,685.2	1,208.5	1,685.2	.....	6.91	5.85	6.91	.....	3.10	3.81	3.70	.....	7.15	5.97	7.23
51	.....	32.5	31.0	32.7	.....	1,800	1,300	1,800	.....	1,685.2	1,208.5	1,685.2	.....	6.91	5.85	6.91	.....	3.44	3.21	3.40	.....	7.42	7.28	8.04

HIGH-NITROGEN RATION

3	22.5	33.1	30.4	33.0	650	2 000	1,850	2,000	600.0	1,838.8	1,710.5	1,849.2	11.82	36.22	34.04	36.80	5.26	6.94	6.76	7.50	3.29	6.36	6.03	7.00
6	23.8	33.5	31.5	33.2	1,600	2,100	1,350	2,200	1,476.8	1,930.7	1,218.2	2,034.1	29.09	38.04	24.84	40.48	6.29	20.38	13.51	16.50	12.25	12.81	8.12	9.69
9	26.7	33.2	31.7	33.7	1,900	2,400	1,000	2,400	1,753.7	2,206.6	924.6	2,219.0	34.55	43.47	18.40	44.16	9.60	21.40	9.18	18.70	9.94	11.12	6.77	11.85
12	28.0	33.9	31.3	34.3	2,400	2,400	2,100	2,400	2,215.2	2,218.6	1,941.7	2,219.0	43.64	41.04	38.64	44.16	12.93	19.41	12.86	19.75	14.52	11.67	7.88	10.63
15	29.1	35.2	32.6	35.1	2,400	2,400	2,400	2,400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	21.38	16.47	20.82	13.38	11.57	12.57	9.06
18	29.9	35.2	34.9	35.3	2,400	2,400	2,400	2,400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.08	20.76	19.25	29.92	12.23	11.95	12.04	14.87
21	30.3	35.8	36.0	35.3	2,100	2,400	2 400	338	2,206.6	2,218.6	2,216.2	312.1	43.47	41.04	38.64	5.44	15.52	22.80	21.91	22.29	13.05	12.75	13.31	5.78
24	30.4	35.6	35.6	34.2	2,400	2,400	2,400	800	2,206.6	2,218.6	2,216.2	738.7	43.47	41.04	38.64	12.88	16.87	23.64	20.80	12.59	14.17	13.85	12.77	5.20
27	30.5	36.0	36.6	35.4	2,400	2,400	2,400	1,400	2,218.6	2,218.6	2,216.2	1,292.8	41.04	41.04	38.64	22.54	17.17	20.75	21.68	13.95	13.60	12.50	14.49	6.52
30	30.7	36.1	37.2	33.6	2,400	2 400	2,400	2,200	2,218.6	2,221.9	2,216.2	2,031.5	41.04	41.10	38.64	35.42	18.44	22.66	20.67	20.06	12.71	10.21	15.13	10.38
33	31.7	35.6	36.9	33.4	2,400	2,400	2,400	2,400	2,218.6	2,221.9	2,231.5	2,231.7	41.04	41.10	38.83	38.83	21.08	22.38	15.94	15.31	14.60	15.35	15.02	12.63
36	32.7	26.5	37.8	34.8	2 400	2,400	2,400	2,400	2,218.6	2,221.9	2,231.5	2,231.7	41.04	41.10	38.83	38.83	23.39	22.80	16.05	25.92	15.63	11.05	13.51	14.16

[1] Lambs 2 and 4 were fed the high-nitrogen corn-gluten meal ration for 18 days, beginning with the eighteenth day.

All lambs consumed the same amount of the high-nitrogen rations during the initial period. Lambs 3 and 4, which were fed the corn-gluten meal ration, excreted slightly more nitrogen in the urine than the other two lambs, which were fed the soybean oil meal ration. However, the difference was significant only in the case of lamb 4. This lamb proved to be less efficient than the other lambs throughout the entire experiment.

As stated previously, the rations fed each of the four lambs were changed abruptly to either the dried skim milk low-nitrogen ration or the corn-gluten meal low-nitrogen ration. Lamb 1 was fed the dried skim milk ration for a period of 36 days. This lamb refused to eat well, especially after the twenty-first day. Because of the low state of nutrition of this lamb and its decided lack of thriftiness,

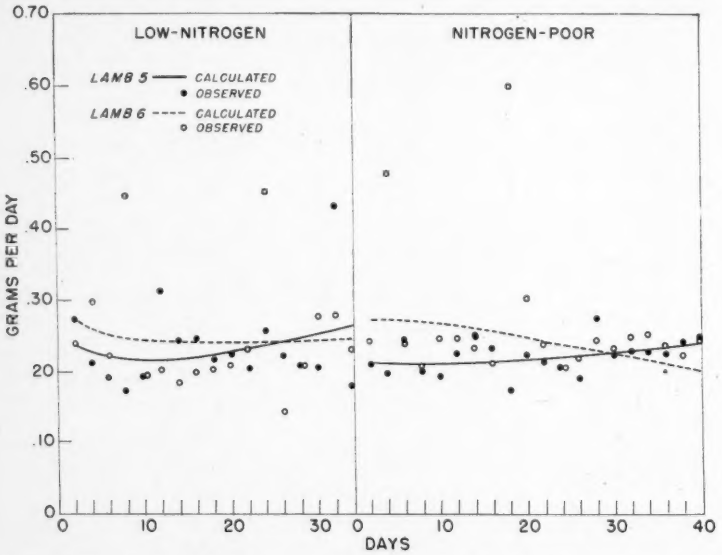

FIGURE 5.—Average daily losses of nitrogen in the urine of lambs when fed the high-nitrogen ration.

this ration was discontinued and the high-nitrogen ration of the next phase of the experiment was fed.

Lambs 2 and 4, which should have been fed the low-nitrogen corn-gluten meal ration throughout this period, were by error fed the high-nitrogen corn-gluten meal ration from the eighteenth to the thirty-sixth day. Immediately following this period during which the high-nitrogen ration was fed, the lambs were again fed the former low-nitrogen ration. Lamb 3 was fed the dried skim milk low-nitrogen ration for 51 days.

The lambs in this experiment reacted much as the lambs in experiment 1 reacted when the low-nitrogen basal ration was substituted for a high-nitrogen ration. The amount of feed the lambs consumed was less by the ninth day or earlier, and they lost weight. These data indicate that the declines in feed consumption and body weight were

not so severe as in experiment 1 when lambs were fed the low-nitrogen basal ration, but were somewhat greater than when the lambs were fed the nitrogen-poor ration containing approximately 4 percent protein.

The excretions of urinary nitrogen decreased rapidly for each lamb until approximately the ninth or twelfth day. After that time no significant trend was evident in the urinary nitrogen excretion by the two lambs which were continued on the low-nitrogen rations. In the case of lambs 2 and 4, a considerable increase in the urinary

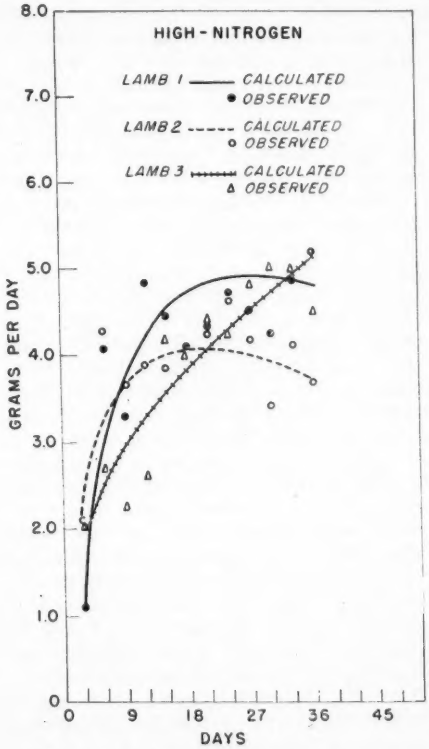

FIGURE 6.—Average daily losses of nitrogen in the feces of lambs when fed the high-nitrogen ration.

nitrogen losses appeared in the first 3-day period following the feeding of the high-nitrogen ration. When the feeding of this ration was discontinued, the lambs again excreted at the low levels of urinary nitrogen. Neither lamb excreted as much urinary nitrogen per day during this 18-day period as during the initial period when the high-nitrogen rations were fed. However, the feed intake was also lower, which would account for much of the difference. The average percentage of nitrogen intake that was excreted in the urine was about the same for the two lambs during the last 9 days as during the initial 9-day collection period.

The fecal nitrogen excretions of the lambs likewise reached a fairly stable level by the ninth to the twelfth day of the low-nitrogen period. From that time on the fecal nitrogen losses appeared to be more closely correlated with feed intake than with the length of time the lambs were fed the low-nitrogen ration.

All lambs regained their appetites fairly promptly when changed to the high-nitrogen ration. The feed was held at the same level that each lamb had consumed of the ration during the first phase of the experiment. All but lamb 4 consumed this amount of feed regularly until the end of the experiment.

Good gains were made by all the lambs when fed the high-nitrogen ration. Lamb 4, however, failed to gain as much as the other lambs, since this lamb went off feed for a few days during the period.

The urinary nitrogen excretions rose when the changes in rations were made. As shown in figure 5, some time was required for the lambs to become adjusted to the higher feed and nitrogen intakes. The urinary nitrogen excretions appeared to become more uniform for each lamb by the time the lambs had consumed the desired amounts of the rations. However, the lambs excreted somewhat less nitrogen in the urine for some time, even after allowing for the lower feed consumption and the lag in urinary nitrogen losses. In order to study this problem further and to remove the effect of slight variations in the nitrogen intake by the lambs, the urinary nitrogen losses were converted into the percentage of nitrogen intake that was excreted in the urine. Only the data for lambs 1, 2, and 3, which completed the 36-day period without going off feed, were considered.

The percentage of the nitrogen intake that was excreted in the urine was extremely variable during the first 9 days. However, as might be expected, the lambs in each case excreted a lower percentage of the nitrogen intake during this 9-day period than during any of the other three 9-day periods. The data for each lamb indicated that a lower percentage of the nitrogen intake was excreted in the urine during the second 9-day period (tenth to eighteenth day, inclusive) than during the third 9-day period (ninteenth to twenty-seventh day, inclusive). Treatment of these data statistically by the analysis of variance method (*18*) shows that this difference is highly significant. These results would indicate that a 9- or 10-day intervening period following the low-nitrogen period is not enough time to enable lambs to regain their adequate consumption and reach a normal state of nutrition. Therefore, nitrogen-balance data obtained under such conditions would result in values for protein utilization that would be too high.

A comparison of the percentage of nitrogen intake excreted in the urine by the lambs during the third 9-day period (nineteenth to twenty-seventh day, inclusive) with that excreted during the fourth 9-day period (twenty-eighth to thirty-sixth day, inclusive) does not show a significant difference on the average. In the case of lamb 1, there was a marked difference, as the nitrogen losses were appreciably greater during the fourth period than during the third period. There was practically no difference in the data for the two periods for lamb 2; for lamb 3, the trend was somewhat reversed.

As shown in table 4, lamb 1 did not eat the low-nitrogen ration as well as the other lambs. Consequently, he lost more weight and entered the high-nitrogen period in a much poorer state of nutri-

tion. Lamb 2 was undoubtedly in the best state of nutrition, as shown by a lower loss of weight during the second phase of the experiment. It is of interest to note that lamb 1 excreted the least urinary nitrogen of the three lambs throughout the high-nitrogen period and apparently did not reach a stable level of urinary nitrogen losses during the course of the experiment. On the other hand, lambs 2 and 3 apparently reached a more or less stable rate of urinary nitrogen excretion sometime during the nineteenth to the twenty-seventh-day period.

With the exception of lamb 2, the lambs failed, in general, to excrete as much nitrogen daily in the urine as they did during the first phase of the experiment when eating the same amounts of the same rations. These data indicate that the lambs were somewhat more efficient in their use of nitrogen as a result of having been previously fed rations low in nitrogen.

The fecal nitrogen losses during this phase of the experiment appeared to be well correlated with the feed intake after the first few days (fig. 6). No definite conclusions can be drawn concerning the relative efficiency with which the lambs digested or utilized the protein of soybean oil meal and corn-gluten meal. The data on the nitrogen excretion for lamb 4 are irregular because of irregular feed intake. While lamb 3, which was fed the corn-gluten meal ration, retained slightly less nitrogen than lambs 1 and 2, which were fed the soybean oil meal ration, the difference could not be considered significant.

EXPERIMENT 3

OBJECT

The object of experiment 3 was to determine the effect that the feeding of a low-nitrogen ration may have on the efficiency with which lambs later retain nitrogen.

PROCEDURE

Nitrogen balance trials with eight growing wether lambs were conducted to study this problem. Four different test rations and a low-nitrogen basal ration were fed. One group of four lambs was used in a series of trials in which two hay rations were compared; the remaining four lambs were fed two rations having either corn-gluten meal or soybean oil meal as nearly the entire source of nitrogen.

These particular rations were used in order to supplement data previously obtained at this station (*10*, *21*). In the earlier experiments, the data indicated a slight superiority of the nitrogen in the one-third alfalfa and two-thirds timothy ration as compared with the alfalfa ration. Also, the nitrogen in the soybean oil meal ration was found to be slightly superior to that furnished by the corn-gluten meal ration.

At the beginning of this experiment each lamb was fed one of the four rations in place of his usual barn ration. Ten days after each lamb was safely on a good level of feeding, nitrogen balance trials were begun.

After each group of four lambs had been fed each of the two rations, the low-nitrogen ration was fed. This change of rations was made abruptly by changing the rations at a morning feeding. On the eleventh day of feeding the low-nitrogen ration the collections for nitrogen balances were started.

Following the low-nitrogen period, each lamb was fed the same experimental ration that he received at the start of the experiment. Again the change in feeding was made abruptly, and collection of excreta was begun on the eleventh day following the change. The order of feeding was also the same as in the first part of the experiment. The rations are shown in table 5.

TABLE 5.—*Percentage composition, and average protein content of rations used in experiment 3*

Ingredients	Soybean oil meal ration	Corn-gluten meal ration	Alfalfa ration	⅓ alfalfa-⅔ timothy ration	Low-nitrogen ration
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Wheat straw	25.0	25.0			25.0
Cellulose	9.0	9.0			10.5
Alfalfa			50.0	16.7	
Timothy				33.3	
Corn			34.0	44.4	
Soybean oil meal	24.3			4.2	
Corn-gluten meal		23.04			
Starch	18.0	18.96	14.0		28.0
Sugar	17.7	18.00			28.0
Corn oil	3.0	3.00	1.0		4.5
Minerals [1]	3.0	3.00	1.0	1.4	4.0
Total	100.0	100.00	100.0	100.0	100.0
Average protein content (N × 6.25)	11.4	11.4	9.5	9.7	1.05

[1] The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

RESULTS

The results of experiment 3 are shown in table 6.

TABLE 6.—*Summary of digestibility and utilization of the nitrogen in the rations fed in experiment 3*

Item	Before low-nitrogen period					After low-nitrogen period				
	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Average	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Average
Alfalfa ration:	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Apparent digestibility	61.7	66.8	66.0	65.5	65.0	55.7	67.0	56.6	65.3	61.2
Total nitrogen retained	29.0	16.0	33.3	18.4	24.2	16.4	20.7	29.5	27.7	23.6
Digestible nitrogen retained	47.0	24.1	50.4	28.0	37.4	29.5	30.9	52.0	42.5	38.7
Biological value	74	58	75	60	67	67	63	79	71	70
Alfalfa ⅓, timothy ⅔:										
Apparent digestibility	65.0	69.6	64.8	67.9	66.8	62.1	69.8	64.8	60.1	64.2
Total nitrogen retained	19.2	30.9	26.2	18.4	23.7	32.7	29.0	31.3	33.4	31.6
Digestible nitrogen retained	29.6	44.4	40.4	27.0	35.4	52.7	41.6	48.3	55.5	49.5
Biological value	62	69	68	58	64	79	68	75	81	76
	Lamb 5	Lamb 6	Lamb 7	Lamb 8		Lamb 5	Lamb 6	Lamb 7	Lamb 8	
Soybean oil meal:										
Apparent digestibility	72.7	71.7	72.1	72.0	72.1	74.6	72.2	65.0	70.5	70.6
Total nitrogen retained	28.5	18.0	29.3	20.1	24.0	28.0	27.6	31.1	24.3	27.8
Digestible nitrogen retained	39.2	25.2	40.7	28.0	33.3	37.5	38.3	47.8	34.5	39.5
Biological value	70	52	62	55	60	68	62	69	61	65
Corn-gluten meal:										
Apparent digestibility	71.9	73.0	69.9	70.6	71.4	71.1	69.5	64.5	68.6	68.4
Total nitrogen retained	25.0	32.9	26.0	20.1	26.0	27.7	29.7	31.9	24.7	28.5
Digestible nitrogen retained	34.8	45.1	37.2	28.4	36.4	39.0	42.7	49.4	36.0	41.8
Biological value	68	67	61	56	63	72	66	72	63	68

The nitrogen contained in the alfalfa ration was digested somewhat less efficiently than the nitrogen contained in the one-third alfalfa two-thirds timothy ration. The data on digestibility obtained previous to the low-nitrogen period check unusually well with data obtained in the earlier experiment in which the alfalfa ration was found to have an apparent digestibility of 64.5 $\pm$0.71 and the one-third alfalfa two-thirds timothy ration an apparent digestibility of 66.7$\pm$0.84. There was no difference in the digestibility of the nitrogen in the soybean oil meal and in the corn-gluten meal rations, although each showed a higher digestibility than either of the two hay rations. In nine comparisons the digestibility after the low-nitrogen period was lower by as much as 1 percent than the digestibility before the low-nitrogen period. In six comparisons there was no difference, and in only one case was the digestibility higher after the low-nitrogen period. A treatment of these data statistically, by Student's method (*18*), shows a high mathematical significance.

The percentage of the total nitrogen retained by the lambs averaged higher following the low-nitrogen period. There were 5 exceptions among the 16 comparisons. A similar statistical treatment of these data shows a mathematically significant difference with odds of 60:1 that the higher values obtained following the low-nitrogen period were not due to chance alone.

There was a somewhat greater difference in the percentage of digested nitrogen retained before and after the low-nitrogen period than in the percentage of total nitrogen stored. This greater difference may be explained by the lower digestibility of the nitrogen following the low-nitrogen period. The odds were approximately 300:1 that the greater retention of digested nitrogen following the low-nitrogen period was not due to sampling error. Also, the biological values computed from these data averaged higher following the low-nitrogen period. The odds were approximately 300:1 and therefore these differences were mathematically significant.

This experiment was so planned that the nitrogen balance trials for two lambs fed each ration were conducted from the eleventh to the twentieth day, inclusive, following the low-nitrogen period, while the trials for the other two lambs fed the same ration were conducted from the twenty-first to the thirtieth day, inclusive. Thus, a comparison was possible on the utilization of the nitrogen between lambs at these two intervals of time following the low-nitrogen period. However, it should be noted that these data are not strictly comparable since different lambs were used in the two periods. There were eight comparisons between these two periods available for study.

It is of interest to note that the nitrogen losses in the urine for those lambs for which data were obtained during the eleventh- to twentieth-day period averaged lower than for those which were on the collection period during the twenty-first- to thirtieth-day period. The average apparent digestibility of the nitrogen in the rations for the earlier period was also lower than for the later period. On the other hand, there was practically no difference in the percentage of total nitrogen stored, the percentage of digested nitrogen stored, or the biological values.

The above data agree well with those obtained in the two earlier experiments. They show that the greatest effect on the urinary

nitrogen losses and the digestibility of the nitrogen occurs just after the low-nitrogen period. However, the effect of the low-nitrogen period undoubtedly influences the utilization of nitrogen by the lambs even after 20 days. In fact, even though the urinary nitrogen excretion may reach a fairly stable level within 30 days, the losses may still be lower for a considerably longer time; in other words, the lambs may be more efficient than if no low-nitrogen period of feeding had occurred.

Treatment of these data mathematically by means of the analysis of variance (*18*) showed no significant difference in the biological values obtained for the alfalfa ration and the one-third alfalfa and two-thirds timothy ration. Furthermore, this treatment of the data indicated no significant difference in nitrogen utilization between these two rations either before or after the low-nitrogen period. Similarly, there was no mathematically significant difference in the biological values for the soybean oil meal ration and the corn-gluten meal ration, either before or after the low-nitrogen period.

As previously mentioned, earlier experiments indicated a slightly lower efficiency of the nitrogen in the alfalfa ration as compared with the one-third alfalfa and two-thirds timothy ration. Since these additional data show no significant difference, it may be assumed that the earlier results were unduly influenced by one especially low value obtained for the alfalfa ration.

Also, earlier experiments involving three lambs had shown a slight superiority of the protein in the soybean oil meal ration as compared with the corn-gluten meal ration. Results obtained in this experiment indicate that the nitrogen of the corn-gluten meal ration was at least fully equal to that contained in the soybean oil meal ration.

GENERAL DISCUSSION OF RESULTS

The three experiments reported in this paper were planned to give specific answers to some of the questions which have arisen during the several years that quality of protein for ruminants has been under intensive study at this station. Although the results obtained apply specifically to the problems which arise when lambs are used, at least some of the same problems would arise with the use of other experimental animals.

The data reported in this paper indicate that a preliminary period of 10 to 12 days is sufficient for lambs to reach a more or less stable level of urinary nitrogen excretion after the feeding of a low-nitrogen ration. Apparently, this length of time is sufficient to take care of the lag in urinary nitrogen excretion which always follows when a marked change is made in the protein content of the ration. However, the authors of this paper are inclined to believe, along with certain other workers, that the urinary nitrogen losses determined during a collection period of sufficient length, even following such a preliminary period, may not necessarily represent the true endogenous losses. However, data have been obtained at this station on a fairly large number of lambs and the values tend to average consistently around a value of 0.037 gm. of endogenous nitrogen daily per kilogram of weight, even though there have been some marked variations within the data. To obtain these values, a low-nitrogen ration has been used, which furnished only the small amount

of protein that was present in the wheat straw which made up 25 percent of the total ration.

The low-nitrogen basal ration which has been used at this station is considered to be only partly satisfactory. It is not devoid of protein, and, at the present time, the authors have little hope of being able to formulate an entirely nitrogen-free ration for lambs that will be sufficiently palatable for use in such experimental work (*8*). Therefore, no direct check is possible to determine whether the small amount of straw protein affects the endogenous nitrogen values or whether the values obtained with this ration truly represent the endogenous level. Lambs fed the low-nitrogen ration containing 25 percent wheat straw are not capable of maintaining good appetites or their body weights. Feed consumption usually becomes irregular. Undoubtedly, some lambs respond so poorly to this ration that their nitrogen losses are not sufficiently reliable to be used safely.

The addition of dried skim-milk protein to the low-nitrogen basal ration in an amount to furnish 4 percent protein makes a more palatable ration. Because of the higher level of feed intake, as well as higher nitrogen intake, the lambs maintain their weights or make slight gains in weight. However, contrary to data obtained with rats (*14*), the addition of this protein does significantly affect the urinary nitrogen losses. The average value obtained from two lambs during the eleventh to twentieth day, inclusive, while fed the low-nitrogen basal ration was 0.0388 gm. per kilogram of body weight per day, while the value obtained from the same lambs and the same corresponding period on the nitrogen-poor ration containing approximately 4 percent dried skim milk protein averaged 0.0474 gm.

The addition of only enough dried skim milk or corn-gluten meal to furnish approximately 1.2 percent protein likewise improved the appetites of the lambs and their body weights. However, in no instance did these lambs have as strong appetites as those fed the 4 percent protein rations, nor could they maintain their body weights. The values for the endogenous nitrogen losses from lambs fed either dried skim milk or corn-gluten meal at the low levels of protein averaged 0.0438 gm. per kilogram of body weight.

There may be several reasons why lambs have responded differently than rats. Lambs are ruminants, and might be expected to respond differently to various proteins and amounts of protein entirely as a result of species difference. Moreover, egg rather than milk protein has been used largely in rations for rats. However, nitrogen balance experiments with lambs at this station (*10*) have shown that dried skim milk protein is as efficient as any common source of protein when compared at a 10-percent level. On the other hand, any particular protein that is of average quality at a 10-percent level of feeding might be utilized nearly 100 percent at a low level. If this is true, the quantity of protein furnished would affect the nitrogen metabolism of the animals more than would the quality. In the experiment reported in this paper, the protein of corn-gluten meal, fed at a 1.2-percent level, was apparently utilized with the same efficiency as the dried skim-milk protein similarly fed, although no conclusions were possible from the limited amount of data available. The data indicate that neither source of protein at either the 1.2-percent or the 4-percent level was completely utilized.

The metabolic nitrogen in the feces appeared to be affected but little by the addition of the small amounts of protein. The fecal nitrogen losses were higher on the average during the periods when skim-milk protein or corn-gluten-meal protein was included in the rations, but likewise the feed intake was greater. On the basis of dry-matter intake, the fecal nitrogen losses were similar to those found when the low-nitrogen basal ration was fed. Apparently, the digestibility of the protein was complete or nearly complete at these low levels.

Evidence was obtained in these experiments that subjecting lambs to a low-nitrogen ration influenced their utilization of nitrogen at a later date. Even though a 10-day preliminary period following the low-nitrogen period was apparently sufficient time to allow for the chief lag in nitrogen excretion following the change to a test ration, the effect of the preceding low-nitrogen ration still existed. These data indicate that the lambs were more efficient in their utilization of nitrogen because of a lower urinary nitrogen loss. On the other hand, there was a small though significant difference in the reverse direction in the digestibility of the protein.

The data indicate further that on the average lambs failed to reach a more or less stable level of urinary nitrogen losses until sometime after 20 days had elapsed. Furthermore, for at least 30 days after a low-nitrogen period, lambs were more efficient in the use of nitrogen than if they had not been subjected to a low-nitrogen period of feeding. For some time following a low-nitrogen ration, lambs apparently utilize nitrogen with an unusually high efficiency in order to regain nitrogen lost from the body, as well as to regain lost body weight. These experiments do not show definitely when this influence ceases to exist, although they indicate that the period of higher efficiency is probably influenced inversely by the length of time the lambs are fed the low-nitrogen ration or to an even greater extent by the feed intake of the lambs and their losses in body weight during this period. On the other hand, if lambs are in a severely low state of nutrition at the close of the low-nitrogen period, they may not respond well to a ration containing ample protein.

The relative value of the nitrogen in one ration as compared with the value of the nitrogen in another is apparently not influenced by a low-nitrogen period of feeding. Therefore, even though the values obtained for the nitrogen utilization following a low-nitrogen period would tend to be higher than could be expected normally, the values would have a comparative significance with others obtained in the same manner, provided the values were obtained after the same length of time had elapsed since the low-nitrogen period.

On the basis of these experiments, the authors believe certain modifications should be made in the former methods used in determining biological values of proteins for lambs. If direct values for endogenous and metabolic nitrogen are to be determined for each lamb, the necessary period of low-nitrogen feeding should be conducted at the end of the experiment and not preceding or during the course of the experiment. Following this period on a low-nitrogen ration, the lambs should be discarded from future experimental use.

Also, the authors believe that average values for the endogenous and metabolic nitrogen, as determined from a sizeable number of

lambs of comparable age and weight, may be fully as desirable to use in calculating biological values as individual values obtained directly for each experimental lamb. In the grouping of data, abnormally low or high values that may be explained on the basis of extremely low feed intake, or other known factors, could be discarded. On the other hand, it is difficult to discard data for an individual lamb in a small experiment, even though the values appear questionable. The variation in values for endogenous and metabolic nitrogen losses which commonly appear are probably due more to the manner in which the lamb responds to the ration, as shown by differences in feed intake, health, and condition of the animal, or loss in body weight, than to the possible intrinsic differences between lambs in their endogenous losses.

The authors prefer to emphasize the percentage of total nitrogen retained and the percentage of digested nitrogen retained rather than biological values in expressing nitrogen utilization by lambs. It is appreciated that biological values accurately determined would give a more quantitative measurement of the utilization of the digested nitrogen. However, the other methods of expressing the efficiency of protein furnish information of as much or more practical value and can be determined for lambs with less experimental error. Fortunately, in the series of experiments conducted at this station the comparative values between rations have been the same whether expressed in terms of the retention of total nitrogen, the retention of digested nitrogen, or biological values. In this connection, it should be pointed out that no matter whether the results are expressed in terms of biological values or of percentages of total or digestible nitrogen retained, it is essential that there be the same percentages of protein in all rations that are compared.

The experiments reported in this paper have furnished information upon the variation within short periods of the urinary and fecal nitrogen excretions of lambs. On inspection of these data, it seems probable that collection periods of considerable length are desirable. There may be a doubt as to whether or not 10-day collection periods are long enough in nitrogen balance studies with lambs.

SUMMARY

Three nitrogen balance experiments have been conducted with growing wether lambs to study certain problems connected with the determination of biological values of protein. Specifically, the major problems studied were: (1) The length of time required by lambs to reach an endogenous nitrogen level when fed a low-nitrogen ration, (2) the effect on feed consumption, on loss in body weight, and on nitrogen excretion of adding a small amount of dried skim milk or corn-gluten meal to the low-nitrogen basal ration, (3) the time required by lambs to reach a stable level of nitrogen utilization when fed rations containing 10 percent or more protein following the feeding of a low-nitrogen ration, and (4) the influence of a low-nitrogen ration on the accuracy of biological value as heretofore obtained with lambs.

The data reported in this paper indicate that a preliminary period of 10 to 12 days was sufficient for lambs to reach a more or less stable level of both urinary nitrogen and fecal nitrogen excretion following

the feeding of a low-nitrogen ration. Although the minimum urinary nitrogen losses may not have been entirely reached within this time, the chief reduction in urinary nitrogen losses had occurred.

The addition of dried skim-milk protein to the low-nitrogen basal ration in sufficient amounts to furnish approximately 4 percent or 1.2 percent protein increased the palatability of the rations and resulted in less loss of weight. However, the urinary nitrogen losses were greater when the dried skim milk was fed at either level than when the basal ration was fed. Apparently, the nitrogen of the dried skim milk was not completely utilized even at these low levels. The results in regard to fecal nitrogen losses indicate that the dried skim-milk nitrogen at each level was completely digested and did not affect the metabolic nitrogen losses.

These experiments show further that feeding lambs a low-nitrogen ration influences the utilization and digestibility of nitrogen at a later date. Lambs were more efficient in their utilization of nitrogen because of a lower urinary nitrogen loss for at least 30 days after the low-nitrogen period of feeding of 20 days. There was a small though significant difference in digestibility, with the protein being less digestible for the lambs following the low-nitrogen period of feeding. However, the relative efficiency of the nitrogen between rations was not influenced when all comparisons were comparable in regard to time following the low-nitrogen period of feeding. The biological values as determined for four rations following a low-nitrogen period of feeding were higher than the biological values determined for these same rations and by the same lambs prior to a low-nitrogen period.

LITERATURE CITED

(1) Ashworth, U. S.
1935. GROWTH AND DEVELOPMENT. ENDOGENOUS NITROGEN AND BASAL ENERGY RELATIONSHIPS DURING GROWTH. Mo. Agr. Expt. Sta. Res. Bul. 223, 20 pp., illus.

(2) ——— and Brody, S.
1933. GROWTH AND DEVELOPMENT. XXVIII. DECLINE OF ENDOGENOUS NITROGEN EXCRETION PER UNIT WEIGHT WITH INCREASING WEIGHT IN GROWING RATS, AND ITS RELATION TO THE DECLINE IN BASAL METABOLISM. DECLINE IN LIVE WEIGHT, NITROGEN, AND ENERGY METABOLISM WITH THE ADVANCE OF THE PERIOD OF NITROGEN STARVATION AND THE INFLUENCE OF LIVE WEIGHT AND OF PRECEDING LEVEL OF PROTEIN INTAKE ON THESE DECLINES AND ON THE SURVIVAL PERIODS. Mo. Agr. Expt. Sta. Res. Bul. 190, 19 pp., illus.

(3) Brody, S., Procter, R. C., and Ashworth, U. S.
1934. GROWTH AND DEVELOPMENT. BASAL METABOLISM, ENDOGENOUS NITROGEN, CREATININE AND NEUTRAL SULPHUR EXCRETIONS AS FUNCTIONS OF BODY WEIGHT. Mo. Agr. Expt. Sta. Res. Bul. 220, 40 pp., illus.

(4) Chick, H., Hutchinson, J. C. D., and Jackson, H. M.
1935. THE BIOLOGICAL VALUE OF PROTEINS. VI. FURTHER INVESTIGATION OF THE BALANCE SHEET METHOD. VII. THE INFLUENCE OF VARIATION IN THE LEVEL OF PROTEIN IN THE DIET AND OF HEATING THE PROTEIN ON ITS BIOLOGICAL VALUE. Biochem. Jour. 29: 1702–1711, 1712–1719, illus.

(5) ——— and Roscoe, M. H.
1930. THE BIOLOGICAL VALUES OF PROTEINS. I. A METHOD FOR MEASURING THE NITROGENOUS EXCHANGE OF RATS FOR THE PURPOSE OF DETERMINING THE BIOLOGICAL VALUE OF PROTEINS. Biochem Jour. 24: [1780]–1782.

(6) McCollum, E. V.
1914. THE VALUE OF THE PROTEINS OF THE CEREAL GRAINS AND OF MILK FOR GROWTH IN THE PIG, AND THE INFLUENCE OF THE PLANE OF PROTEIN INTAKE ON GROWTH. Jour. Biol. Chem. 19: 323–333.
(7) Martin, C. J., and Robison, R.
1922. THE MINIMUM NITROGEN EXPENDITURE OF MAN AND THE BIOLOGICAL VALUE OF VARIOUS PROTEINS FOR HUMAN NUTRITION. Biochem. Jour. 16: [407]–447, illus.
(8) Miller, J. I.
1937. EFFECT OF PROTEIN DEFICIENCY IN THE RATION ON THE AMOUNT OF FEED CONSUMED BY LAMBS. Jour. Agr. Res. 55: 467–474, illus.
(9) ——— and Morrison, F. B.
1938. THE NUTRITIVE VALUE FOR FARM ANIMALS OF THE PROTEINS FURNISHED BY COMMON PROTEIN-RICH SUPPLEMENTS AND BY TYPICAL LEGUME AND NONLEGUME RATIONS. N. Y. (Cornell) Agr. Expt. Sta. Rpt. (1938) 51: 91.
(10) ——— and Morrison, F. B.
1939. RELATIVE EFFICIENCY FOR GROWING LAMBS OF THE PROTEIN IN RATIONS CONTAINING ALFALFA HAY, TIMOTHY HAY, AND COMBINATIONS OF THE TWO HAYS. Jour. Agr. Res. 58: 149–155.
(11) ——— Morrison, F. B., and Maynard, L. A.
1937. RELATIVE EFFICIENCY FOR GROWING LAMBS OF THE PROTEIN IN RATIONS SUPPLEMENTED BY SOYBEAN-OIL MEAL, LINSEED MEAL, OR CORN-GLUTEN MEAL. Jour. Agr. Res. 54: 437–448.
(12) Mitchell, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. Jour. Biol. Chem. 58: 873–903.
(13) ——— Burrows, W., and Beadles, J. R.
1936. THE SIGNIFICANCE AND ACCURACY OF BIOLOGICAL VALUES OF PROTEINS COMPUTED FROM NITROGEN METABOLISM DATA. Jour. Nutr. 11: 257–274.
(14) ——— and Carmen, G. G.
1926. THE BIOLOGICAL VALUE OF THE NITROGEN OF MIXTURES OF PATENT WHITE FLOUR AND ANIMAL FOODS. Jour. Biol. Chem. 68: 183–215.
(15) ——— and Hamilton, T. S.
1931. THE NUTRITIVE VALUE FOR GROWING SWINE OF THE PROTEINS OF LINSEED MEAL AND OF COTTONSEED MEAL, BOTH ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN. Jour. Agr. Res. 43: 743–748.
(16) Schneider, B. H.
1935. THE SUBDIVISION OF THE METABOLIC NITROGEN IN THE FECES OF THE RAT, SWINE, AND MAN. Jour. Biol. Chem. 109: 249–278, illus.
(17) Smuts, D. B.
1935. THE RELATION BETWEEN THE BASAL METABOLISM AND THE ENDOGENOUS NITROGEN METABOLISM, WITH PARTICULAR REFERENCE TO THE ESTIMATION OF THE MAINTENANCE REQUIREMENT OF PROTEIN. Jour. Nutr. 9: 403–433.
(18) Snedecor, G. W.
1938. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 2, 388 pp., illus., Ames, Iowa.
(19) Sotola, J.
1930. BIOLOGICAL VALUES AND SUPPLEMENTARY RELATIONS OF THE PROTEINS IN ALFALFA HAY AND IN CORN AND SUNFLOWER SILAGE. Jour. Agr. Res. 40: 79–96.
(20) Turk, K. L., Morrison, F. B., and Maynard, L. A.
1934. THE NUTRITIVE VALUE OF THE PROTEINS OF ALFALFA HAY AND CLOVER HAY WHEN FED ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN. Jour. Agr. Res. 48: 555–570.
(21) ——— Morrison, F. B., and Maynard, L. A.
1935. THE NUTRITIVE VALUE OF THE PROTEINS OF CORN-GLUTEN MEAL, LINSEED MEAL, AND SOYBEAN-OIL MEAL. Jour. Agr. Res. 51: 401–412.

U. S. GOVERNMENT PRINTING OFFICE: 1942